Psychoendocrine Correlates of the Healthy Human Pregnancy

Thesis (cumulative thesis)
Presented to the Faculty of Arts and Social Sciences
of the University of Zurich
for the Degree of Doctor of Philosophy

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Accepted in the Fall Term 2017 on the Recommendation of the Doctoral Committee:
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Zurich, 2018

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Acknowledgements

First and foremost, I would like to thank most sincerely my supervisor Professor Ulrike Ehlert. Professor Ehlert not only launched the project, on which this thesis is based on, she also guided me with her great expertise, encouragement, and deep knowledge throughout the time of the Ph.D. Professor Ehlert always provided support by directing me through the, sometimes challenging, phases of the past three and a half years. I am overly grateful for being part of her research team and for her advice, flexibility, optimism and the freedom she gave me. I consider it a true honour to have worked with Professor Ehlert.

Secondly, I would like to express my gratefulness to Professor Roland Zimmerman for being my second supervisor. My deepest appreciations for taking the time to read and evaluate this thesis and providing feedback for the scientific work along the way. In addition, my special thanks goes to Professor Christian Breymann, who also took the time to read and evaluate this thesis.

My special thanks goes to Dr. Pearl La Marca-Ghaemmaghami, who not only additionally supervised the research project, but also personally invested herself into it. She supported me throughout the Ph.D. time as a personal mentor and always provided me with instrumental and emotional support. Her door was always open for supportive advice and for finding solutions.

Further, I would like to thank the Swiss National Science Foundation, which financially supported the project, upon which this thesis is based on. The flexibility and long-sightedness of this institution is very much appreciated.

In addition, my appreciation goes to the study participants for the valuable time they provided by participating voluntarily in the research project. Without them, it would have not been possible to conduct this project and to write the current thesis.

I would also like to thank the students and research interns, who invested their time and effort to successfully conduct this project. The study team stood out by being overly dedicated

and enthusiastic and my appreciation goes to the Aurelia Keller, Sandra Angst, Tatjana Bickel, Hannah Süss, Michelle Heuberger and Alessia Corso.

Next, I would like to thank all the colleagues and friends at the Department of Clinical Psychology and Psychotherapy at the University of Zurich. The great atmosphere within the team created a truly enjoyable work place. My fellow Ph.D. students always provided instrumental and emotional support and contributed to this time as a great experience. Their initiative and helpfulness truly inspired me. I would like to express my deep appreciation to Niclà Lozza, who provided patiently methodological support. My heartfelt thanks goes to my former colleague, dear friend and office companion Dr. Patricia Waldvogel and my very dear friend Eszter Pataky. Without them, I would have missed countless wonderful moments and experiences along the way. It was a true pleasure to work within our team. Further, I would like to especially thank my long-term friends Nadine Weymerskirch, Lina Sulzbacher and Lena Pruess. They always offered emotional and instrumental support. Having countless discussions throughout the process, they tolerated my tensions and made me feel like everything is gonna be alright.

There are no words to describe the deep gratitude I feel towards my parents Susanne and Claus Hunold. They stood behind me throughout my entire courses of studies in two foreign countries and spared no efforts to support me. Their warmth, kindness and constant believe in my abilities enabled me to never give up. They heartily advised and guided me through all the ups and downs, letting me know that there is always a solution. I am very proud and thankful to have such wonderful parents, who I consider my personal role models. I would have not achieved this without them. I dedicate this dissertation to my parents.

I also would like to express my special thanks to my siblings Lennart and Lisa Hunold. These two accompanied me throughout my entire live and during the time of the Ph.D. always constituted a source of emotional support. Their laughter and joy were welcome distractions and gave me new energy to carry on. Further, my immense gratitude goes to my grandparents, Hildegard and Fred Sparenberg, for their steady emotional support and their friendly ear, whenever I needed it.

Acknowledgements

My partner, Pascal Schaad, often put things into perspective and constituted a calm anchor for me while I was writing this dissertation. He truly dedicated himself to support me throughout the stressful times and was always there for me, when I needed him. He provided me with countless joyful moments and always positively encouraged me. He absorbed my moods and insecurities on a daily basis and encountered it with his endless optimism and consolation. My very special gratitude is addressed to him. Thank you for being a truly wonderful partner and for sharing your live with me.

Abstract

The present thesis attempted to contribute to a better understanding of the determinants of the psychoendocrine correlates of a healthy human pregnancy. Therefore, the predictive values of various psychological parameters of stress with regard to maternal wellbeing and birth outcome were examined. To address the underlying endocrine mechanisms of naturally occurring stress, stress hormones were investigated over the course of gestation. This thesis unites two empirical studies, which were conducted to explore the potential correlation of psychological stress and maternal and foetal health, as well as the development of cortisol and cortisone over the course of pregnancy.

In the first empirical study, the associations of several psychological work related aspects of stress during pregnancy and maternal wellbeing and birth outcome were investigated. Previous research demonstrated negative associations of work related strains during pregnancy and maternal wellbeing and birth outcome. To be more precise, quantitative aspects of work stress, like long working hours or shift work, were found to predict adverse health outcome in the mother and the child. Nevertheless, psychological aspects of work stress were mainly overlooked and research appeared very limited. The first study aimed at extending these findings to psychological work stress determining maternal wellbeing and birth outcome, expecting a negative association. One hundred singleton pregnant women completed an online questionnaire in early pregnancy and the third trimester to assess work stress. Maternal wellbeing was assessed in early pregnancy and the early postpartum period and neonatal birth parameters were gathered from the medical records after delivery. These parameters were analysed in the total study population and two subsamples, namely in women with high job strain and in women with low income. In conformity with the hypotheses, aspects of psychological work stress were associated with lowered maternal wellbeing and unfavourable neonatal birth outcome in all study groups, with the subsample analyses revealing an additional association for neonatal birth outcome. These findings contribute to a better understanding of

which psychological work related aspects of stress strain maternal wellbeing and potentially affect the health of the foetus.

In the second empirical study, the glucocorticoids cortisol and cortisone, assessed in saliva, were investigated over the entire course of gestation and the early postpartum period. The cortisone-cortisol ratio, which was additionally examined, is assumed to represent the enzymatic activity of 11β-hydroxysteroid dehydrogenase type 2 in the parotid glands. The metabolic function of the enzyme is assumed to constitute a natural barrier for maternal over-glucocorticoid exposure. In previous research, cortisol and cortisone were found to progressively rise from approximately mid gestation. Also, the enzymatic activity is presumed to increase with ongoing pregnancy. Yet, these results were obtained by mainly considering single point measures within one or two pregnancy trimesters. We therefore examined the glucocorticoids and their ratio in four week intervals in 100 singleton pregnant women over the course of gestation, starting in early stages of pregnancy and leading into the early postpartum period. We found that salivary cortisol and cortisone levels rose from 17-20 weeks` gestation and that the awakening responses of the glucocorticoids displayed the same increase but further revealed decreases at postpartum. The analysis of the ratio exhibited a decline between 17 and 24 gestational weeks and increased from 33 gestational weeks. These findings confirm an increase in the hormones from approximately mid gestation onwards. However, it was also shown that the enzymatic activity, and its concomitant metabolic function, appears reserved in the second pregnancy trimester and upregulated in the third pregnancy trimester. This might lead to the assumption of the second pregnancy trimester constituting a time period of a potentially elevated risk for glucocorticoid over-exposure.

To conclude, the present thesis extends the knowledge about psychobiological factors and their associations during pregnancy. The novel insights contribute to a deeper knowledge about the determinants of psychoendocrine parameters during human gestation.

Figures

Tables

Abbreviations

11β-HSD	11β-hydroxysteroid dehydrogenase
11β-HSD1	11β-hydroxysteroid dehydrogenase type 1
11β-HSD2	11β-hydroxysteroid dehydrogenase type 2
ACTH	Adrenocorticotropic hormone
AME	Apparent mineralocorticoid excess
AUC	Area under curve
AUCg	Area under curve relative to ground
AUCi	Area under curve relative to increase
AVP	Arginine vasopressin
BMI	Body mass index
CAR	Cortisol awakening response
cDNA	Complementary deoxyribonucleic acid
CBG	Corticosteroid binding globulin
CHF	Swiss franc
CRH	Corticotropin releasing hormone
CRH-BP	Corticotropin releasing hormone binding protein
EPDS	Edinburgh Postnatal Depression Scale
GR	Glucocorticoid receptor
HPA	Hypothalamus-pituitary-adrenal
ICC	Intra-class correlation coefficient
IUGR	Intra uterine growth restriction
MR	Mineralocorticoid receptor
NADP	Nicotinamide adenine dinucleotide phosphate
pCRH	Placental corticotropin releasing hormone
PVN	Paraventricular nucleus

Abbreviations

SAM	Sympathetic-adrenomedullary
SGA	Small for gestational age
TICS	Trier Inventory of Chronic Stress
TSST	Trier Social Stress Test

1 Introduction

The conceptualization of the stress response in humans developed over decades, however even nowadays, a general definition seems apparently missing. Psychobiological research often utilizes the concept of allostasis to address the definition of stress (Sterling & Eyer, 1988). The authors postulated the necessary adaptability of organisms to environmental changes. The interplay of psychological and physiological mechanisms in response to stressors highlights the mutual consideration in stress research.

The main goal of this thesis was to investigate the psychoendocrine stress response during human gestation. Even in healthy pregnancies, psychosocial stress can develop, due to the wide variety of alterations, which are accompanied with becoming a parent (Ehlert, Sieber, & Hebisch, 2003). Maternal stress during pregnancy is assumed to be associated with impacted maternal wellbeing and adverse foetal health parameters (Stanton, Lobel, Sears, & DeLuca, 2002). The concept of foetal programming assumes that the maternal stress physiology can structurally and functionally alter foetal organs during critical periods of gestation, which in turn may lead to long-lasting health conditions in the offspring (Barker, 2007). This demonstrates the essentiality to further explore correlates of the maternal psychoendocrine stress response. The maternal hypothalamus-pituitary-adrenal (HPA) axis undergoes drastic changes over the course of gestation, with the naturally elevated secretion of corticotropin releasing hormone (CRH), andrenocorticotropic hormone (ACTH) and cortisol (Meinlschmidt & Heim, 2003; Wadhwa, Sandman, Chicz-DeMet, & Porto, 1997). In addition, an interaction of the maternal and foetal stress systems develops.

The empirical examination of the maternal stress response upon a stressor during pregnancy was mainly analysed with regard to ACTH and cortisol levels in late stages of pregnancy and revealed mixed results (e.g., Gitau, Fisk, Teixeira, Cameron, & Glover, 2001). Scientific results regarding the second trimester of pregnancy appear very limited, but revealed that this phase might be vulnerable with regard to a psychoendocrine stress response (e.g.,

Ghaemmaghami, Dainese, La Marca, Zimmermann, & Ehlert, 2014). Research considering the first trimester seems to be limited as well. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is attributed to play a critical role during gestation, as it is presumed to constitute a natural metabolic barrier, which functions to guard the unborn child from maternal glucocorticoid over-exposure. The enzyme is located in various bodily tissues, like the parotid glands, and during gestation it can be additionally found in the placenta (Chapman, Holmes, & Seckl, 2013). The development of the expression of 11β-HSD2 over the course of pregnancy seems ambiguous, as scientific investigations revealed contradicting results. It was mainly assumed to elevate with progressive gestation, however also oppositional outcomes were found (e.g., Shams et al., 1998; Kajantie et al., 2003). Longitudinal investigations appear to be missing though, as mostly only two to three measurement points over the course of pregnancy were compared with each other.

Maternal stress, anxiety and depressive values during gestation seem to impact the expression and activity of 11β-HSD2 (O`Donnell et al., 2012). A reduction of 11β-HSD2 was repeatedly correlated with impacted birth outcome parameters and pregnancy related illnesses (Schoof et al., 2001a; Stewart, Rogerson, & Mason, 1995). It is postulated that 11β-HSD2 can also be measured in maternal saliva by examining the ratio of cortisone and cortisol (Meulenberg & Hofmann, 1990). However, the truly investigation of salivary 11β-HSD2 over the course of pregnancy seems to be likewise missing.

Human pregnancy is not only characterized by various physiological changes, but also by psychosocial modifications, which can elicit a stress experience. Chronic stress during pregnancy is associated with allostatic load and is presumed to affect maternal wellbeing and birth outcome (Dunkel Schetter & Tanner, 2012). One factor contributing to chronic maternal stress over the course of gestation is work related stress. A wide variety of external work related factors (e.g., night shift) was shown to be related with adverse birth outcome (e.g., Katz, 2012), even though the results were not consistent throughout empirical investigations. In comparison, psychological aspects of work stress (e.g., work overload) were studied to a far lesser extent.

1 Introduction

The focus was laid on birth outcome parameters, with the consequences of work stress for maternal wellbeing appearing strongly understudied.

This thesis firstly aims at amplifying the knowledge with regard to various psychological aspects of work related stress and its predictive value for maternal wellbeing and birth outcome parameters. Secondly, the glucocorticoids cortisone, cortisol and their ratio, assessed in saliva, were repeatedly investigated over the course of gestation, beginning in early pregnancy up to the early postpartum period. The examination of the development of these glucocorticoids constitutes an additional goal of this thesis. The thesis is segmented into three major parts. Part I constitutes a theoretical background of empirical research with regard to the human psychoendocrine stress response during human pregnancy. From the empirical findings, the current research questions were derived and study hypotheses were formulated. The second part includes the presentation of the findings of the two empirical studies. These are summarized and critically discussed in detail in the third part.

PART I:

THEORETICAL BACKGROUND

2 The Stress Response in Humans

The first section of the thesis addresses the human stress response from a psychobiological perspective. The chapter starts with the exposition of psychological and biological reactions towards adverse stimuli, with an ensuing detailed presentation of the endocrine stress reaction. With reference to empirical research and theoretical frameworks, the focus is put on various impacts of stress on the human psychology and physiology.

2.1 The Psychobiological Stress Response

In ordinary language, as well as in scientific communication, a universal definition of the concept of stress appears to be still missing (Monroe, 2008). The applicability of Selye`s quote (1973, p. 692) *"everybody knows what stress is and nobody knows what it is"* still seems contemporary. Stress research developed over decades and the concept of stress was repeatedly adapted. As laid out in the review article by Ramsay and Woods (2014), Claude Bernard (1870/1973) emphasised, that it is critical for an organism, in order to function optimally, to sustain a stable intra-physical environment. This would allow for an ideal execution of physiological processes, independently of changes in the environment and of interferences. Bernard coined the expression *milieu intérieur*, which implies that the physiological regulatory mechanisms aim at sustaining vitality, despite challenging environmental confrontations.

In 1929, Cannon introduced the term *homeostasis* to demonstrate the interaction between two bodily systems, namely the sympathetic nervous system and the adrenal medulla, which attempt to restore balance when the organism is faced with negative sensations, such as fear or pain. Homeostasis is obtained, when an organism is confronted with adverse stimuli, by the activation of the sympathetic nervous system and the release of epinephrine by the adrenal medulla. This activation serves to fight or flee in dangerous situations. The relevance of the situations is assessed by emotional evaluation (Cannon, 1914). A negative feedback is the

response of the organism and secures homeostasis (Rosenblueth, Wiener, & Bigelow, 1943). Cannon (1932) proposed that homeostasis is a physiological and behavioural reaction towards perturbing physiological stimuli.

Selye (1956) conceptualized stress in physiological and biomedical terms. He stated that either physiological or psychological adverse stimuli, called stressors, elicit a non-specific bodily reaction towards it. Further, in relation to the concept of a non-specific response to a stressor, he introduced the *general adaptation syndrome*, which consists of three stages: alarm, resistance, and exhaustion (Selye, 1950). The organism experiences an imbalance, which involves an enhanced activation of the sympathetic nervous system (alarm stage). During the stage of resistance, the parasympathetic nervous system tries to antagonize this imbalance. If the stressor does not occur repeatedly or continuously, the organism can be placed again into homeostasis. However, if the stressor appears over a prolonged time or in high intensity, an imbalance between the sympathetic and parasympathetic nervous system can emerge, which describes, according to Selye (1976), the stage of exhaustion. The author also differentiated between distress and eustress. Distress defines stress effects, which hold negative consequences. Eustress, on the other hand, describes positive consequences of stress, for example when an organism is positively challenged (Selye, 1976). Although, Selye is often entitled as the originator of the stress concept (Viner, 1999), the concept of a non-specific physiological response towards a stressor appears obsolete nowadays. Physical and psychological adverse stimuli seem to provoke diverse reactions and not every individual perceives the same stimulus as stressful.

According to Levine and Ursin (1991), the stress conceptualization should entail a stimulus, the perceptual processing of that stimulus, and the physiological and behavioural response towards or upon the stimulus. Lazarus and Launier (1981) consider a stress response as a dynamic interaction between a person and its environment. The authors pursue a cognitive phenomenological approach to define psychological stress by its appraisal. To encounter this approach, Lazarus and Folkman (1984) published the *transactional model of stress*. Firstly, this model involves the stage of primary appraisal, which describes the cognitive appraisal of a

situation. Personality factors seem to be involved in the process, if a certain situation is perceived as potentially harmful, threatening or positively challenging. The secondary appraisal defines the evaluation of the potential coping strategies. Based on the primary and secondary appraisal and the interpretation of the situation, stress emotions can be elicited. According to Lazarus and Folkman (1984), these can individually vary in intensity and motion. Following these stages, coping is initiated. Coping is considered as adaptive when, either the situation itself is constructively managed, or the emotional attitude towards the situation is changed. This is also called reappraisal. If the assessment of coping strategies is considered as insufficient, stress is experienced. Hence, the transactional model of stress emphasizes the individual perception of a situation or a stimulus as potentially stressful.

However, particular events seem to elicit a stress response in almost every individual. Psychological stressors (e.g., uncontrollability, novelty, unpredictability and threats with potential harm or loss) can be considered as enormously potent of causing a physiological response in the endocrine system (Dienstbier, 1989; Henry & Grim, 1990; Mason, 1968; Rose, 1980). Stressors can be characterized in terms of their intensity and the required time of adaptation. The following types of stress can be differentiated: daily hassles, chronic stress, critical life events and traumatic events (Perrez, Laireiter, & Baumann, 2011). Daily hassles are considered as relatively minor interfering and frustrating events, like train cancellations on commute. The concept of chronic stress requires a stressor to persist over an enduring period. For example, a persistent excessive demand at work is considered as a chronic stressor. Critical life events, on the other hand, are stressors, which imply serious consequences, like the death of a closely related person. Traumatic events are considered to elicit a strong and long-lasting stress response in almost every individual, which leads to desperation. To witness a natural disaster or to be a victim of violence represent traumatic events (Perrez et al., 2011).

The concept of stress has been redefined in recent times. Nowadays, it is assumed, that in the case of repeatedly appearing or continuing stress, the adaptive strategies could be overburdening. In this case, homeostasis might not be maintained and the result might be a steady physiological stress reaction, which can have adverse consequences for the organism

(Hobel, Goldstein, & Barrett, 2008). To address this assumption, Sterling and Eyer established in 1988 another conceptualization of stress. The model of *allostasis* is determined, according to the authors, by achieving stability throughout change. In order to adjust to environmental changes, organisms must adapt their regulatory mechanisms (Sterling & Eyer, 1988). As the concept of homeostasis, developed by Cannon (1929), does not take learning and the anticipation of reactions into account, Sterling and Eyer (1988) amplified the stress concept. For the purpose to sustain homeostasis and to ensure enduring survival, allostasis is characterized by adaptation to imminences and to variances in the environment of an organism (Peters & McEwen, 2012). The term allostatic load was introduced to consider the emerging costs of an organism, to sustain a regulatory mechanism, which is constantly replaced from its former function (McEwen & Stellar, 1993). Allostatic load, with regard to its compensatory mechanisms, can define long-term effects of stress and can constitute the transition to illness (Birbaumer & Schmidt, 2010; McEwen, 1998). Allostatic load can be evoked by four different mechanisms. Firstly, the repeating experience of a stressful situation, secondly the inability to adapt to these recurrent demands, thirdly the inability to fully recover from the stressful experience and finally the emergence of a destructive allostatic regulatory mechanism (McEwen, 1998).

This section displayed the theoretical frameworks of the interaction of psychological and biological reactions when an organism is confronted with a stressor and it demonstrated that also psychological stressors induce a physical reaction. It was outlined that the stress conceptualization developed and adapted over decades. Cannon (1929) established the term homeostasis to demonstrate the body`s attempt to preserve a balance when the organism is confronted with physical or psychological challenges. Selye (1956) postulated a non-specific physiological reaction upon adverse stimuli. However, the non-specific response theory from Selye was adapted and the aspect of stimuli appraisal was considered. Lazarus and Folkman (1984) developed the transactional model of stress, which accentuates the individual perception of stimuli and their reactions upon it. In 1988, the concept of allostasis was developed by Sterling and Eyer and they stated that the human body has to adapt to environmental changes

in order to adjust. Allostatic load develops, when the adjustment is insufficient and the organism compensates for these deficiencies. The endocrine stress reaction is described in depth in the following.

2.2 The Endocrine Stress Response

Within this section, the stress conceptualization is considered in physiological terms. The focus in this paragraph is put on the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic-adrenomedullary (SAM) system. The systems of the HPA axis and SAM have their origin in the hypothalamus (Gunnar & Quevedo, 2007). Brain stem and cortico-limbic structures are activated, when a stressor is faced (Ulrich-Lai & Herman, 2009). The sympathetic and autonomic nervous system constitute together with the adrenal medulla, the SAM, which is relevant for an immediate stress response (Ulrich-Lai & Herman, 2009). The SAM, as being a part of the sympathetic branch of the autonomic nervous system, consists of sympathetic preganglionic neurons (McEwen, 2000). These neurons appear in the spinal cord and activate the cells in the medulla of the adrenal glands (Gunnar & Quevedo, 2007). According to the authors, these cells release the hormones epinephrine and norepinephrine. Within seconds, an immediate stress response is initiated by the SAM, through the regulation of the organ activity. This leads to a reduction of the blood pressure and skin resistance, a constriction of the blood vessels, an increase of the heart rate, and an activation of the muscular system and by this prepares the organism for a flight or fight reaction (Birbaumer & Schmidt, 2010; Ulrich-Lai & Herman, 2009). The activation of the rapidly acting SAM is also called first wave stress response and the slow acting HPA axis can be referred to as second wave response, which is described in more detail in the following section. If a stressor persists after the initial sympathetic activation, this stimulation is maintained until the nerve cells of the hypothalamus are reached and the HPA axis is activated (Kaluza, 2012).

2.2.1 Regulation of the Hypothalamus Pituitary Adrenal Axis

The hypothalamus and the pituitary gland constitute a unit, which is super-ordinated towards the endocrine system (Hick & Hick, 2009). It describes a neuro-endocrine hormonal axis, which synthesizes and secretes various steroid hormones (Paschke & Voigt, 2010). The HPA axis is activated when an organism experiences stress and conduces to provide cortisol as an end-product for metabolic processes (Deutzmann, 2012). The HPA axis is organized in a hierarchical manner and when activated, releases two peptides, namely corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) from neurons of the paraventricular nucleus (PVN) inside the hypothalamus (Kaltsas & Chrousos, 2007; Paschke & Voigt, 2010). This stimulates the release of the adrenocorticotropic hormone (ACTH) in the anterior lobe of the pituitary gland (Hick & Hick, 2009). When the organism does not face an acute stressor, the secretion of CRH and AVP follows a circadian rhythm. The pulsatile manner, in which CRH and AVP are released, rises in the early morning and leads to ACTH, and finally cortisol secretion (Engler et al., 1989; Horrocks et al., 1990). In stressful situations, the pulsatile secretion of CRH and AVP is enhanced, which induces an increase of ACTH, as well as of cortisol release (Tsigos & Chrousos, 1994). ACTH arrives at the cortex of the adrenal gland via bloodstream, where the production and secretion of the glucocorticoid cortisol is stimulated (Ehlert, 2011). Cortisol secretion peaks within 10 to 30 minutes after onset of the stressor (Zoccola, Quas, & Yim, 2010). Cortisol is considered to be involved in the regulatory mechanisms of the bodily homeostasis, as well as the biological response towards stress (Tsigos & Chrousos, 2002). By a negative glucocorticoid feedback mechanism, ACTH secretion is constrained, to prevent a limitless duration of glucocorticoid exposure (Kaluza, 2012; Tsigos & Chrousos, 2002). The HPA activity is inhibited by cortisol on the pituitary, as well as on the hypothalamic level, whereby the two receptors glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) are assumed to be critically involved (Ehlert, 2011). Cortisol binds primarily to MR and to a lesser extend to GR (Gunnar & Vazquez, 2006). When the organism is exposed to an acute or temporary stressor, the HPA axis elicits an adaptive stress

response (Kaluza, 2012). However, chronic stress can lead to an impairment of the mechanisms of the HPA axis, by an elevated cortisol release leading to a decreased response of ACTH to exogenous CRH. Physical, as well as psychological long-term or repeatedly occurring stressors, can impact the HPA axis. This in turn influences psychological and biological health functioning (Dickerson & Kemeny, 2004). Cortisol is seen as a primary indicator of biological alterations as a consequence of stress exposure (Kirschbaum & Hellhammer, 1989). The glucocorticoid cortisol and its critical part in the stress response will be discussed in more detail in the following section.

2.2.2 Cortisol

The glucocorticoid cortisol, as the final product of the HPA axis, follows naturally a circadian rhythm and is released in a pulsatile fashion (1-2 secretions per hour) (Weitzman et al., 1971). The peak is reached in the early morning, followed by a constant decrease and displaying lowest levels in the middle of the night (de Weerth, Zijl, & Buitelaar, 2003). The diurnal profile of cortisol develops in early infancy (Gunnar & Vazquez, 2006). The daily decline of cortisol is conceptualized as a marker of the negative feedback mechanisms of the HPA axis and the potential to rally from daily stress (Dmitrieva, Almeida, Dmitrieva, Loken, & Pieper, 2013). A wide variety is demonstrated by the impact of cortisol on physiological processes. It is involved in the regulation of metabolic processes, by an enhancement of the glucose levels in the blood stream. Hereby, cortisol stimulates the metamorphosis of amino acids and other substrates into glucose. These processes proceed in the liver and allow the breakdown of fat cells and proteins, which displays an exposition of energy reserves (Dickerson & Kemeny, 2004). Further, the functions of the central nervous system, inflammatory system or the vascular reactivity are affected by the glucocorticoid (Halpern, Whitsel, Wagner, & Harris, 2012). In addition, cortisol displays an inhibiting effect on parts of the immune system. It blocks proteins, which regulate inflammatory processes (Dickerson & Kemeny, 2004). After cortisol enters the bloodstream, 90 to 95 % is bound to proteins, namely corticosteroid binding globulin (CBG) and albumin

(Kirschbaum & Hellhammer, 1999). The remaining minority of cortisol is so called free or unbound cortisol, which is distributed in the blood and is mainly responsible for the physiological reactions (Ekins, 1990). The unbound cortisol can cross body and brain cells, where corresponding receptors are activated and these enter the cell nucleus, where the gene transcription is impacted (Gunnar & Quevedo, 2007). Next to these genomic effects, cortisol can also have non-genomic effects, by acting upon membrane receptors (Wehling, 1997). As unbound cortisol cannot only be measured in blood, but also in saliva, the latter has become the favoured measurement method in research.

Cortisol enters saliva by passive diffusion and appears uninfluenced by the salivary flow rate. Correlations of the unbound form of cortisol in blood and saliva are found to be high, which enables the use of salivary cortisol for physiological stress assessment (Hellhammer, Wüst, & Kudielka, 2009; Kirschbaum & Hellhammer, 1994). According to Kirschbaum and Hellhammer (1994), cortisol, measured in saliva, can be even more associated with representing the unbound cortisol fraction in blood plasma and serum than the total cortisol serum level. These high correlations persist during the circadian rhythm (Hellhammer et al., 2009). It is assumed that the allostatic load has an influence on the diurnal cortisol activity (McEwen & Stellar, 1993). These findings enable the use of saliva samples for the assessment of cortisol in research, which can be collected in a non-invasive and low-risk manner in comparison to the plasma or serum cortisol assessment, which requires venepuncture. This procedure of non-invasive sampling is convenient for research studies with a great number of participants, as participants can autonomously sample their own saliva at every time and place. There are various methods by which saliva can be sampled. One of the mostly used sampling devices is the *Salivette* (Sarstedt Inc., Rommelsdorf, Germany). Participants chew on a cotton swab, which is returned into a centrifugation tube. For saliva sampling in infants, a cotton dental roll can be used, with which the mouth is swabbed (Kirschbaum & Hellhammer, 1994). Another method constitutes passive drooling using *SaliCaps* sampling devices (SaliCap, IBL International GmbH, Hamburg, Germany). The non-invasive and easily applicable sampling devices for saliva collection allows to investigate hormones in a natural setting.

Through these simplified procedures, the psychoneuroendocrinological examination of the *cortisol awakening response* (CAR) received increasing attention during the past decades. The CAR is defined as an indicator of the acute reagibility of the HPA axis and also follows a diurnal pattern (Fries, Dettenborn, & Kirschbaum, 2009; Schmidt-Reinwald et al., 1999). The regulating mechanisms of the CAR was described by Pruessner and colleagues (1997) and is characterized by the natural elevation of the cortisol release in the time of 30 to 40 minutes upon awakening with a 50 to 156% salivary cortisol increase (Clow, Thorn, Evans, & Hucklebridge, 2004; Elder, Wetherell, Barclay, & Ellis, 2014). More precisely, the dynamic of the secretion of cortisol upon awakening is referred to as CAR (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010), and the persistent decrement of cortisol during the day is referred to as daily decline (Kirschbaum & Hellhammer, 1989). These two concepts are associated, but constitute comparatively independent processes (Edwards, Clow, Evans, & Hucklebridge, 2001). A relatively high intra individual stability for the CAR is assumed (Fries et al., 2009). The CAR can be operationalized and measured by the *area under the curve* (AUC) relative to ground (AUCg) and relative to increase (AUCi). Relations with regard to health status were found. For example, a decreased CAR was reported to predict an adverse course of anxiety and depressive disorders and further, associations with chronic stress were described (Duan et al., 2013; Vreeburg et al., 2013). Duan and colleagues (2013) found, that participants with elevated perceived stress and anxiety displayed reduced cortisol levels at 30 minutes upon awakening. According to Chida and Steptoe (2009), a generally elevated CAR is associated with the actual experience of psychological stressors and a decreased CAR with unfavourable health outcomes. However, regulatory mechanisms exist in the human physiology to shield the organism from negative effects of stress reactions. In the following section, the potential protective mechanisms of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) are displayed.

2.3 The Conversion of Cortisol and Cortisone

The aforementioned binding of cortisol to the intracytoplasmic MR and GR serves the function to regulate effects of the glucocorticoid in human organs. However, if an organism is confronted with a chronic stressor, these receptors might be over-stimulated. To inhibit this effect, cortisol is reversibly transformed into cortisone by the enzyme 11β-HSD (Wyrwoll, Holmes, & Seckl, 2011). The activity of 11β-HSD was first encoded by Carl Monder. In a rat liver, he purified and isolated the cDNA for this enzyme. (Agarwal, Monder, Eckstein, & White, 1989). 11β-HSD contains glucocorticoid inactivating and regenerating activities, with the co-substrate nicotinamide adenine dinucleotide phosphate (NADP) (Chapman et al., 2013). 11β-HSD is classified as a short chain alcohol dehydrogenase and is involved in the mechanisms of transmitting specificity upon the MR (Stewart, Whorwood, & Mason, 1995). Further, 11β-HSD enables cortisol to access the GR in various tissues. In humans, two isoforms of the enzyme were detected. Type 1 11β-HSD (11β-HSD1) is located in the central nervous system, in neurons and glia. Within the hippocampus, cerebellum and neocortex, high expressions of 11β-HSD1 were reported. This form of the enzyme regenerates active glucocorticoids from their inactive form and is assumed to play a critical role in regulating the negative feedback mechanisms of the HPA axis (Wyrwoll et al., 2011). While 11β-HSD1 is found to be involved in converting cortisone into cortisol (Walker, Campbell, Fraser, Steward, & Edwards, 1992), type 2 11β-HSD (11β-HSD2) converts the bioactive cortisol into the overly inactive form cortisone (Agarwall, Mune, Monder, & White, 1994; Stewart et al., 1995). In Figure 1, the metabolic processes of 11β-HSD are displayed.

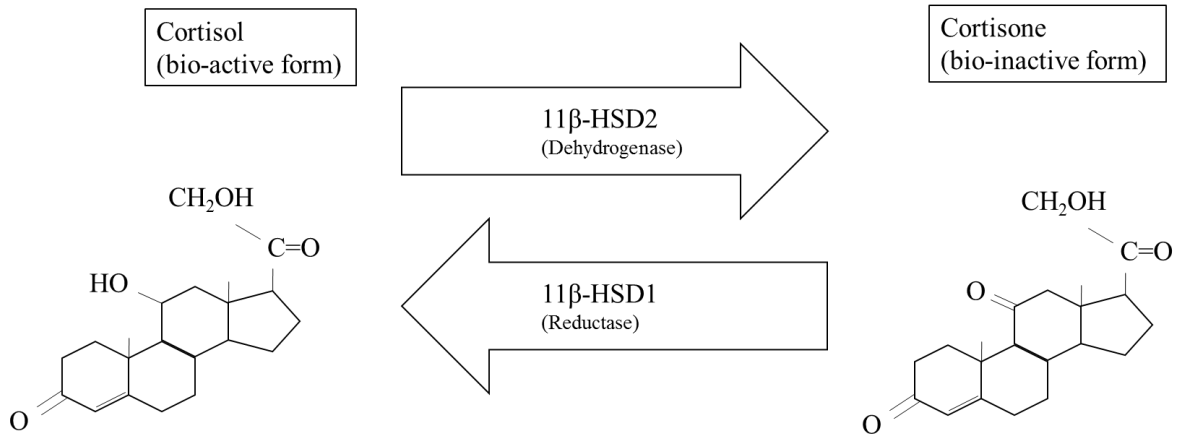

Figure 1: Enzymatic regulations of 11β-HSD are displayed in rodents. 11β-HSD1 metabolises cortisol in cortisone and 11β-HSD2 exhibits the opposing transformation of cortisol into cortisone (adapted from: Wyrwoll et al., 2011).

On the one hand, 11β-HSD is assumed to express intracrine effects. The stimulation of intracellular receptors is regulated by 11β-HSD2, without modifying cortisol levels, which circulate in the blood stream (Leckie, Chapman, Edwards, & Seckl, 1995). On the other hand, endocrine effects are displayed by the turnover of the majority of glucocorticoids. This can affect the HPA functioning. Further, the enzymes within a cell, which alter the negative feedback mechanisms, could by this also alter the circulating cortisone and cortisol levels (Edwards, 2012). The metabolic mechanisms of 11β-HSD2 were detected within various tissues like the kidney, vascular smooth muscle cells, colon and placenta. Further, the type 2 of the enzyme was found within cells of the salivary glands (Smith et al., 1996). It is assumed that 11β-HSD2 holds a key role in the corticosteroid metabolism in comparison to 11β-HSD1, as it is localised on the endoplasmic reticulum within cells, with a cytosol facing active site and a co-factor binding domain, which enables 11β-HSD2 to bind about 100 times more to its substrates than its antagonism (Wyrwoll et al., 2011). As Römer and colleagues (2009) pointed out, the general activity of glucocorticoids does not exclusively depend on the cortisol release, but also on the metabolic functioning, on the obtainability of the cortisol binding globulin, GR

and MR. When an organism experiences stress, the activity of 11β-HSD1 is assumed to be elevated, as an adaptive mechanism of processing the stressor (Quinkler & Stewart, 2003).

However, when the activity of 11β-HSD is altered, adverse health consequences can result. For example, 11β-HSD1 was found to be associated with brain functioning in age. A correlation of an increase of this type 1 enzyme with progressing age was connected to cognitive decline (MacLullich et al., 2010). In adipose individuals, an elevation of 11β-HSD1 expression in subcutaneous adipose tissue was detected, which could display an association of 11β-HSD1 regulation and adipose tissue depot-specific mutations (Rask, et al., 2001). With regard to 11β-HSD2, the *apparent mineralocorticoid excess* (AME) describes a genetically determined insufficient metabolism of cortisol into cortisone, which was linked to perilous physiological consequences (Monder et al., 1986). The resulting deficiency in 11β-HSD2 activity was found to predict mineralocorticoid excess and was related to hypertension, hypokalaemia, prolonged half-life of cortisol and also intrauterine growth retardation, short stature, thirst, polyuria and mutated postnatal growth (Quinkler & Stewart, 2003; Römer et al., 2009).

The ratio between cortisone and cortisol, assessed in saliva samples, can be referred to as a biomarker for the assessment of salivary 11β-HSD2 activity (Perogamvros et al., 2009). The application of this ratio constitutes an implemented method, which was frequently used in assessing 11β-HSD2 activity. It will be further described in section 3.

2.4 Summary of the Psychoendocrine Findings

The conceptualization of stress and the response upon it developed over a long period of time. Physiological and psychological aspects were considered, as well as their interaction. Nowadays, it is assumed that adverse physiological or psychological stimuli elicit an individual response on a cognitive, emotional and biological level. The two physiological stress systems SAM and HPA axis regulate the physiological stress reaction. The SAM, functioning as the first wave stress response, ensures a quick stress response by releasing epinephrine and norepinephrine. These hormones allow a rapid energy mobilization and thereby prepare the

organism for a promptly reaction towards the stressor. The second wave stress response is coordinated by the HPA axis and as an end product, cortisol is released about 30 minutes after the onset of the stressor. Cortisol has genomic and non-genomic effects, which demonstrates the variety of its regulatory mechanisms to maintain homeostasis. The examination of the CAR became an implemented marker for the "acute reagibility of the HPA axis". Saliva samples for the assessment of cortisol are widely used in psychoneuroendocrine research, due to its applicability and non-invasiveness and it is utilized as a marker to investigate psychological and physiological illnesses. However, the focus in stress research was also put on the protective physiological mechanisms, namely on 11β-HSD. 11β-HSD1 converts cortisone into cortisol and by this plays a key role in the negative feedback mechanism of the HPA axis. 11β-HSD2, on the other hand, performs the reverse conversion of cortisol into cortisone and protects the body from a cortisol over-exposure. The assessment of the salivary cortisone and cortisol ratio is assumed to display the 11β-HSD2 activity in the salivary glands and represents an established biomarker. The following chapter focusses on human pregnancy. The psychoendocrine stress response during gestation are described.

3 The Psychoendocrine Stress Response during Pregnancy

Pregnancy is generally perceived as a positive event in the course of a woman's life in western society. However, a pregnancy by itself implies a huge number of modifications towards the expectant motherhood. The necessary adaptations towards pregnancy can provoke a stress experience, when they are not sufficiently accomplished (Pattison & Gross, 1996). Sometimes even the very onset of a pregnancy can elicit stress (Stoz, 1997). The bodily constitution of the pregnant woman undergoes a variety of changes on an anatomic and functional level. This can also be considered as threatening and potentially leading to the experience of stress (Ghaemmaghami, 2011). Consequently, even a healthy pregnancy can be accompanied by psychosocial stress (Bindt, Huber, & Hecher, 2008; Ehlert, 2004). Stress during pregnancy can be assessed differentially. It appears essential to consider environmental stressful stimuli, as well as the response towards it on a cognitive, emotional, behavioural and biological level (Dunkel Schetter & Glynn, 2011). Henceforth, first the psychological aspects of the stress response during pregnancy are described, followed by the description of the endocrine stress response.

3.1 The Psychological Stress Response during Pregnancy

Anxiety, insecurity and reduced wellbeing are mainly associated with the first trimester of pregnancy, not only because of the pending adaptation towards the various psychophysical changes, but also because the elevated risk for abortion during this period and pregnancy related symptoms, such as morning sickness (Bindt et al., 2008). During the second trimester of pregnancy, the psychological and physical wellbeing of pregnant women seems less constrained, as pregnancy symptoms generally decline, foetal movements are perceptible and adaptation towards motherhood advances (Bindt et al., 2008; Vingerhoets & van Dessel, 2003).

3 The Psychoendocrine Stress Response during Pregnancy

The third trimester constitutes the end phase of the pregnancy. This phase can elicit anxiety towards the impeding birth and the new role of the women as mother (Riecher-Rössler & Steiner, 2005). Further, women in their third trimester of pregnancy have an increased girth, which constrains mobility and is often the cause of physical pain, mainly back pain (Gruber, 2009). Moreover, financial and occupational restrictions are often accompanied with having a child, which could lead to further anticipated psychosocial stress during the third trimester (Ehlert et al., 2003). This demonstrates that even during a pregnancy without complications, the experience of stress can evolve. Research found that the majority of pregnant women experience low to moderate psychosocial stress (Woods, Melville, Guo, Fan, & Gavin, 2010). This topic is insofar relevant, as not only the wellbeing of the expectant mother might be compromised, but also because maternal stress was associated with adverse birth outcome (Stanton et al., 2002). Depressive mood during pregnancy was often reported to be associated with shortened gestational length and lowered birth weight (Evans, Heron, Patel, & Wiles, 2007; Woods et al., 2010). Moreover, elevated maternal stress levels during pregnancy were associated with various adverse conditions regarding the child. Among them were an increased risk of abortion (Wainstock, Lerner-Geva, Glasser, Shoham-Vardi, & Anteby, 2013), malformations of the foetus (Tegethoff, Greene, Olsen, Schaffner, & Meinlschmidt, 2011) or problems on an emotional and behavioural level of the child (O'Connor, Heron, Golding, Beveridge, & Glover, 2002). Further, the subjective wellbeing of pregnant women was found to predict their wellbeing postpartum and by this appears critical for developing psychological illnesses, like postnatal blues, depression or psychosis (Dietz et al., 2007). As maternal prenatal stress can elicit an increase in stress hormone levels, which in turn can also affect the foetus, the endocrine stress response during pregnancy will be discussed in depths in the following section.

3.2 The Endocrine Stress Response during Pregnancy

The postulation that the maternal psychoendocrine stress responses during pregnancy can have a long-lasting impact on the child and its development recently became of great interest in research and is called *foetal programming*. The concept of foetal programming contains the assumption that enduring structural and functional alteration of the developing foetal organs are affected by the maternal stress psychophysiology. This has the potential to lead to lifelong health conditions (Barker, 2007). However, the biological mechanisms of foetal programming are not fully understood up to today. One hypothesis is that an overexposure of maternal cortisol is involved in foetal programming (Harris & Seckl, 2011). Another approach, described by Glover (2014), considers an elevated trans-placental passing of maternal cortisol. Hereby it is assumed, that the placental enzyme function of 11β-HSD2 is altered. If the function of placental 11β-HSD2 is reduced, due to chronic overexposure of cortisol, more maternal cortisol can be transported into the foetal compartment. Even though the underlying mechanisms of foetal programming remain partly elusive, research revealed that during gestation, the endocrinology of women changes drastically and can in addition be potentially altered by the experience of stress.

The HPA axis is strongly affected by a pregnancy and elevates its hormone release with gestational age. CRH, ACTH and cortisol concentrations significantly increase during gestation (Meinlschmidt & Heim, 2003). Further, a complex interaction between the maternal and foetal stress systems evolves (Ehlert et al., 2003). CHR is not only produced maternally, but also in the placenta, which is called placental CRH (pCRH) and released into the maternal-foetal unit. To be more precise, pCRH is produced in the placenta chorion, amnion and decidua (Warren & Silverman, 1995). Because the placental unit produces the majority of CRH during gestation, it is assumed that pCRH is represented in maternal plasma CRH concentrations. Further, CRH produced in the hypothalamus, seems not to be able to pass the blood brain barrier (Riley & Challis, 1991). CRH levels in the maternal blood start to increase from gestational week nine (Riley & Challis, 1991). The production of pCRH is induced by maternal and foetal cortisol

secretion. As mentioned above, the negative feedback mechanisms of cortisol upon the regulation of the HPA axis usually prevent the limitless secretion of CRH and ACTH. However, during pregnancy, this feedback mechanism is reversed and displays a stimulating function on pCRH (Lindsay & Nieman, 2005). Because of the positive feedback mechanism of cortisol, pCRH release is elevated, which leads to an overall increased CRH level (Ehlert et al., 2003). ACTH release is stimulated by pCRH in a cascade manner, which than leads to a rise in maternal and foetal cortisol release (Wadhwa et al., 1997). ACTH levels significantly increase in a less rapid manner and only reach the upper levels of the ordinary, non-pregnant levels (Perkins et al., 1995). From about eleven weeks of gestation, maternal cortisol levels in the bloodstream rise. A two- to three-fold increase of cortisol levels were detected and at 25 weeks of gestation, this elevation is also present in cortisol, assessed in saliva samples (Allolio et al., 1990). The free plasma cortisol is assumed to be well represented by the cortisol in saliva, as aforementioned, it represents the unbound and active fraction of the glucocorticoid. Within the second half of the pregnancy, the CRH binding protein (CRH-BP) in maternal plasma binds to pCRH, by this has an inactivating effect, and simultaneously protects the maternal and foetal HPA axis from over stimulation of ACTH and cortisol. However, it is presumed that pCRH has a stimulating effect on the ACTH release of the maternal pituitary, which could cause mild hypercortisolism during pregnancy (Wadhwa et al., 1997). Another hypothesis regarding the rise in total cortisol levels, laid out by de Weerth and Buitelaar (2005), states that this phenomenon might be a consequence of the constrained sensitivity of the hypothalamic pituitary unit towards the cortisol feedback. It might also be explained by an increase in AVP, which stimulates the release of ACTH, which in turn stimulates the cortisol release (Scott, McGarrigle, & Lachelin, 1990). The CRH-BP concentration rapidly decreases about 30 days prior to delivery and induces an increase in free pCRH circulation in the maternal unit (Linton et al., 1993). During labour, CRH levels peak and reach a thousand-fold concentration in comparison to non-pregnant women (Stalla et al., 1989). Maternal CRH is assumed to constitute a biomarker for regulatory mechanisms, which stimulates delivery (Ehlert et al., 2003; McLean

et al., 1995). Despite the drastic endocrinological changes, the circadian rhythm of ACTH and cortisol is assumed to be maintained over the course of pregnancy (Lindsay & Nieman, 2005).

Considering the radical endocrinological changes during pregnancy, the actual stress response to either acute psychological (e.g., cognitive tests) or physiological (e.g., cold or noise) stressors has been studied with regard to the reactivity of the HPA axis, often considering ACTH levels and the end product cortisol. Results regarding the association of cortisol increase and a psychological or physiological stressor appear to be mixed. As presented in the review article from de Weerth and Buitelaar (2005), a study found no significant increase in cortisol levels, assessed in saliva samples, among pregnant women in late pregnancy (gestational week 37) and postpartum in response to a cold pressure test (Kammerer, Adams, Castelberg, & Glover, 2002). However, the non-pregnant control group displayed a significant increase in salivary cortisol levels. Another study conducted by Saisto, Kaaja, Helske, Ylikorkala and Halmesmaki (2004) again found no significant increase in serum cortisol levels in response to a cold pressure test in late pregnancy (gestational week 37) and postpartum. However, the authors reported a two-fold increase in serum ACTH levels for both measurement points. No increase in serum ACTH and cortisol was found in response to a white noise test in women with advanced pregnancy (gestational week 38) (Hartikainen-Sorri, Kirkinen, Sorri, Anttonen, & Tuimala, 1991). According to de Weerth and Buitelaar (2005), a blunted stress reactivity might be present in pregnant women towards acute stressors. Another study supports this hypothesis, where no cortisol alteration was found in pregnant women (gestational week 22 to 35) when receiving a foetal blood transfusion (Gitau et al., 2001). However, mostly large standard deviations were reported, which implies, according to the authors, a huge variety in the magnitude of participants stress reactions. Further, the physiological stress assessment towards either physiological or psychological stressors was mainly conducted during late stages of pregnancy, which displays insufficient investigation of the stress reaction over the entire course of pregnancy. One study investigated the stress response of pregnant women towards an amniocentesis in the second trimester, by analysing cortisol levels, assessed in saliva samples, before, during and after this potentially stressful event. It was found that salivary cortisol

increased significantly upon the stressor (Ghaemmaghami et al., 2014). In another study, cortisol levels, assessed in saliva samples, remained elevated after pregnant women in their second trimester, in comparison to women in their third trimester, conducted a Trier Social Stress Test (TSST), indicating an extended stress recovery. It was concluded, that the second trimester might be a more vulnerable phase in comparison to the third trimester in terms of stress recovery and could also display a critical phase, where an increased cortisol exposure can result (Nierop et al., 2006). In black women from the U.S. with high levels of cumulative stress (interpersonal violence, community violence, discrimination and other negative life events) cortisol levels, assessed in saliva samples and gathered at about 25 weeks of gestation, were found to be higher upon awakening in comparison to those having lower cumulative stress levels (Suglia et al., 2010). Further, elevated cortisol levels upon awakening, assessed in saliva samples within the second trimester (23 weeks of gestation), were found in women delivering early at 36 weeks of gestation (Entringer, Buss, Andersen, Chicz-DeMet, & Wadhwa, 2011). Moreover, lower birth weight and shorter body length was found in neonates of mothers with increased cortisol levels in early pregnancy (13-18 weeks of gestation). In late pregnancy (35-37 weeks of gestation), this association was only maintained for body length of the neonate (Bolten et al., 2011). As mentioned above, one hypothesis of foetal programming states that an over-exposure of maternal cortisol is involved in this mechanism, which could lead to long lasting impairment in physical and psychological health of the offspring. This is why the investigation of more specific regulatory mechanisms of cortisol, like the examination of the CAR, seems significantly important.

The assessment of the CAR during pregnancy, as a biomarker of cortisol physiology, has also received increasing scientific attention. De Weerth and Buitelaar (2005) showed in their study, that the CAR was maintained during pregnancy. The authors investigated cortisol levels, assessed in saliva samples, in women in their third pregnancy trimester (gestational week 32) and also found day to day stability of the CAR. Entringer and colleagues (2010) validated these results. The CAR during pregnancy appears to be generally elevated in comparison to non-pregnant study groups. This is explained by increased basal cortisol levels (Entringer et al.,

2010). Moreover, data from Bolten and colleagues (2011), gathered from pregnant women in 13-18 and 35-37 weeks of gestation, depicted a significant rise in cortisol levels, assessed in saliva samples, upon awakening in early and late pregnancy. However, maternal psychological stress was not found to be associated with cortisol release in this study. Entringer and colleagues (2010) examined pregnant women at 17 and 31 weeks of gestation with regard to their response towards the TSST. Although again, no significant associations of cortisol and the TSST were found, they showed rising cortisol concentrations upon awakening over the course of pregnancy. This increase was characterised by a platykurtic and less steep rise as gestation advanced, displaying an extenuated CAR in later pregnancy. Further, negative correlations between the AUCg CAR (36 weeks of gestation) and cortisol responses, assessed in saliva samples, upon the TSST at eight weeks postpartum were detected (Meinlschmidt, Martin, Neumann, & Heinrichs, 2010). Giesbrecht, Campbell, Letourneau and Kaplan (2013) investigated cortisol in saliva samples in 82 pregnant women. This study considered all three trimesters (measured at three time points on two consecutive days) and analysed the CAR cortisol and diurnal profile with regard to psychological distress. The authors also found an increase in salivary cortisol levels upon awakening over the course of gestation. Interestingly, the authors also reported a decrease in CAR cortisol, however this effect revealed no significance. Another study analysed the CAR with regard to a cognitive behavioural group intervention in pregnant women (weeks of gestation: 10-15) with elevated stress, anxiety and/or depression (Richter et al., 2012). These values were all subclinical. Results showed a smaller CAR at the post-treatment measurement. The authors concluded an attenuated stress reaction in the intervention group.

The maternal CAR during pregnancy was associated with perinatal birth outcome. Entringer and colleagues (2011) found 13 % higher cortisol levels, assessed in saliva samples, upon awakening in women who delivered their children at 36 weeks of gestation in comparison to women delivering their children at 41 weeks of gestation. These findings seem to support the assumption that a naturally attenuated stress response might be present during the third trimester of pregnancy, however within early pregnancy, this effect was not revealed. As maternal stress

generally cannot be prevented for the duration of pregnancy, the biological protective mechanisms of 11β-HSD2 over the course of gestation are described in the following.

3.3 The Conversion of Cortisol and Cortisone during Pregnancy

Glucocorticoids are assumed to have an impact on foetal growth, with elevated levels showing a restrictive effect and a concomitant preparation of the foetus to survive outside the maternal womb (Chapman et al., 2013). The enzyme 11β-HSD2 is presumed to play a key role in the regulatory mechanisms regarding cortisol exposure of the foetus during pregnancy, by converting active cortisol into its inactivated antagonist cortisone. By this, 11β-HSD2 represents a metabolic boundary, which protects the foetus from excessive cortisol exposure. Among the aforementioned tissues, like the parotid gland, 11β-HSD2 is also expressed in the placenta during pregnancy. As laid out in the review article by Chapman and colleagues (2013), during the middle of the 20th century, high levels of cortisone were observed in placental tissues and an elevated 11β-HSD2 activity was found (Osinski, 1960). As displayed in Figure 2, the transformation of maternal cortisol into cortisone was assumed to proceed, before it approached the umbilical cord and by this the foetus (Pasqualini, Lowy, Albepart, Wiqvist, & Diczfalusy, 1970). Maternal cortisol levels were found to be 5 to 10 times higher than in the foetal unit, which seem to confirm this hypothesis (Beitins, Bayard, Ances, Kowarski, & Migeon, 1973).

Within the placenta, 11β-HSD2 activity is verified from the first trimester (Sun, Yang, & Challis, 1997). Mainly, the placental expression of 11β-HSD2 can be located within the syncytiotrophoblast. Research shows contradicting results with regard to the 11β-HSD2 activity over the course of pregnancy. An elevation during late stages of pregnancy was reported, when the majority of maternal cortisol is converted into cortisone (Benediktsson, Calder, Edwards, & Seckl, 1997). Also, Shams and colleagues (1998) detected less placental enzyme activity in samples from 8-12 and 13-20 weeks of gestation in comparison to at term samples. Schoof and colleagues (2001a) validated these results. The authors analysed placental 11β-HSD2 at around

28 and 39 weeks of gestation and found a significant increase in the enzyme activity with ongoing pregnancy. Oppositional, Kajantie and colleagues (2003) found placental 11β-HSD2 activity to decrease with progressing pregnancy. Further, it was shown that around 14 days prior to delivery, the placental 11β-HSD2 activity regresses (Murphy & Clifton, 2003).

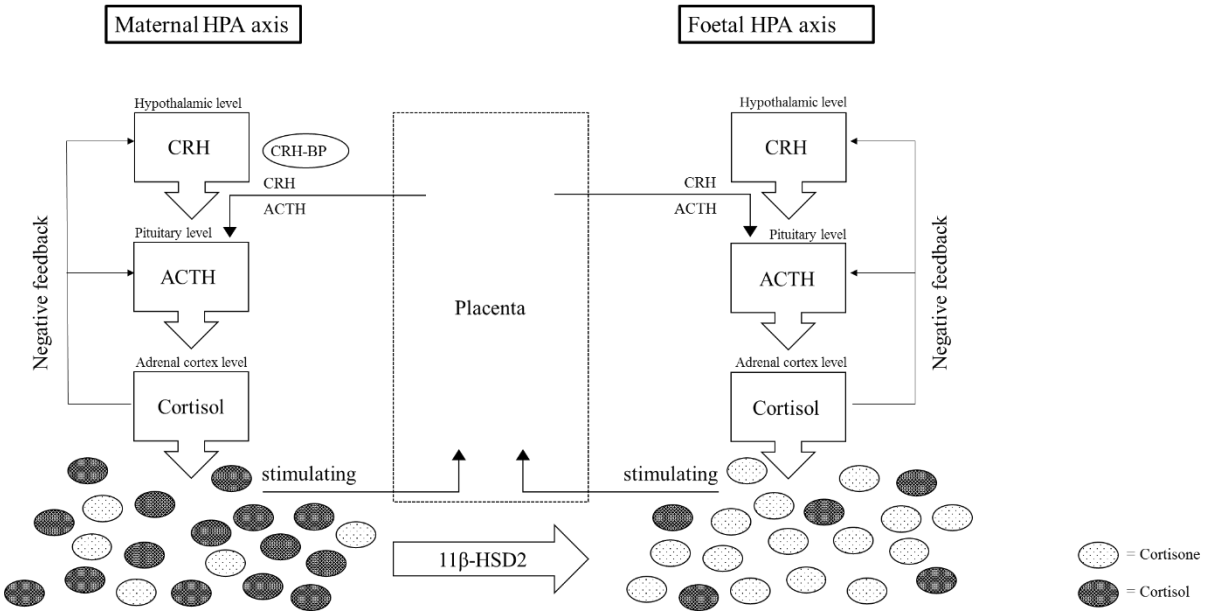

Figure 2: Interplay of maternal and foetal HPA axis with the placental 11β-HSD2 as a natural barrier to prevent the foetus from overexposure of maternal glucocorticoids (adapted from La Marca-Ghaemmaghami & Ehlert, 2015).

Even though, the exact development of 11β-HSD2 over the course of gestation still remains pending, the enzyme expression and activity was found to be linked to maternal stress. The mechanisms of placental 11β-HSD2 seem to be affected by perinatal maternal chronic stress and anxiety and the protective effect of the enzyme might be dampened by the experience of these adverse conditions. In rats, anxiety and prolonged stress experience can contribute to maternal elevated cortisol levels, which in turn can lead to a foetal cortisol exposure (Mairesse et al., 2007). The authors showed a correlation of declining 11β-HSD2 activity and gene expression in stressed pregnant rats. Although, the endocrine mechanisms during pregnancy

differ in rodents and humans, scientific research in humans demonstrated a possible enzyme impairment attributable to stress and anxiety. A stronger association of cortisol levels in maternal amniotic fluid was present in pregnant women with higher anxiety, in comparison to those with lower anxiety levels (Glover, Bergman, Sarkar, & O'Connor, 2009). In line with this, it was found that 10-20% of the maternal plasma cortisol can cross the placenta and increase amniotic cortisol levels by two-fold (Gitau, Cameron, Fisk, & Glover, 1998). O`Donnell and colleagues (2012) showed a negative association of placental 11β-HSD2 expression and maternal prenatal trait and state anxiety, as well as depressive values. Placenta samples were taken from 56 healthy pregnant women with an elective caesarean section. The authors observed a 30% reduction in the enzyme expression. Further, the authors reported the same results for 11β-HSD2 activity in a subsample (N = 25). It was concluded, that elevated maternal anxiousness could affect the permeability of the placenta with regard to cortisol, and by this attenuate the 11β-HSD2 expression. This could explain the elevated foetal cortisol levels with a concurrent absence of enhanced maternal cortisol levels in anxious or stressed pregnant women (O`Donnell et al., 2012). An altered function of placental 11β-HSD2 was correlated with reduced birth weight in normal pregnancies (Stewart et al., 1995). Further, the serious pregnancy related conditions of preeclampsia (Schoof et al., 2001a) and intra uterine growth restriction (IUGR) (Shams et al., 1998) were associated with a reduced expression of placental 11β-HSD2. In a further study, which examined pregnancies with IUGR, the expression of the enzyme was reported to be reduced by 25% (McTernan et al., 2001). Kajantie and colleagues (2003) reported in their study a positive correlation of the neonatal birth weight and 11β-HSD2 activity rate in the placenta. Schoof and colleagues (2001b) also reported a positive predictive value of 11β-HSD2 gene expression for placental weight, as well as neonate birth weight. Over the course of gestation, the 11β-HSD2 expression was also postulated to rise within the parotid glands (Meulenberg & Hofman, 1990). Assumptive due to the 11β-HSD2 expression, levels of cortisone increase exceed cortisol increase in the parotid glands. As cortisone levels, assessed in saliva, rise 2 to 3 folds over gestation, cortisol only elevates its level upon 1 to 2.5 folds (Meulenberg & Hofman, 1990). The authors attribute this proportion to the activity of 11β-

HSD2 in saliva. In the above mentioned study conducted by Ghaemmaghami and colleagues (2014), also salivary 11β-HSD2 was investigated indirectly in 34 pregnant women undergoing an amniocentesis. The authors found a significant decrease in the ratio cortisone/(cortisone+cortisol), which is assumed to represent the 11β-HSD2 activity in saliva, as response to the stressful event of an amniocentesis. Further, it was found that an increased ratio was positively related to neonatal size at birth and weight at birth.

3.4 Summary of the Psychoendocrine Findings during Pregnancy

As described above, even a healthy and normal pregnancy without complications can elicit stress experiences in the expectant mother, due to socioeconomic and physiological changes that accompany pregnancies. The experience of stress can have not only serious psychological and physiological consequences for the maternal wellbeing, but also long lasting consequences for the unborn child. Maternal anxiety and depressive mood during pregnancy were found to predict postpartum conditions like postpartum blues and depression, as well as postnatal health consequences for the child. Reduced gestational lengths and decreased birth weight, and long-lasting emotional and behavioural problems in the child were found to be possible consequences of prenatal maternal stress. The concept of foetal programming assumes that the maternal stress physiology during critical time windows over the course of gestation affects the development of the child. Even though the underlying mechanisms of this concept remain at least partly elusive, it is hypothesized that either the maternal hormone cortisol and the expression and activity of the enzyme 11β-HSD2 play a key role in this process. During gestation, the maternal HPA axis undergoes dramatic changes. It is predominantly assumed that CRH, ACTH and cortisol increase with the progressing pregnancy. Further, CRH is not only produced maternally, but also within the placental unit and a pregnancy determined positive feedback mechanism of cortisol on CRH and ACTH stimulates the secretion. Even though the increase of CRH, ACTH and cortisol during pregnancy develops differentially, elevations proved to be

significant. CRH levels peak shortly before delivery and is therefore considered to play a key role in the onset of birth. The stress response in pregnant women was often analysed with regard to ACTH and cortisol levels and research revealed mixed results. It was shown, that considering the three pregnancy trimesters separately appears to be critical, as stress related results seem to differ with respect to these. Especially, the second trimester seems to be a more vulnerable phase in comparison to the third trimester. The maternal stress response was also assessed with respect to the CAR over gestation. Due to the naturally rise in basal cortisol levels during pregnancy, the CAR was also elevated but maintained. The relation of the CAR and maternal wellbeing and birth outcome variables were likewise inconsistent in comparison to ACTH and cortisol assessment. Elevated cortisol levels upon awakening were detected in women who gave birth in 36 weeks of gestation in comparison to those delivering their child at 41 weeks of gestation.

The enzyme 11β-HSD2, which is hypothesised to possibly also play a role in foetal programming, has received scientific attention over the past decades. Among various tissues, like the parotid glands, 11β-HSD2 during pregnancy is present within the placenta, acting as a natural barrier to protect the foetus from an over-exposure of maternal glucocorticoids. Mainly it assumed that placental 11β-HSD2 expression rises over the course of gestation. However, one study found a converse effect. Maternal stress, elevated anxiety and depressiveness seem to negatively affect the 11β-HSD2 expression and thereby increase the risk of prenatal foetal cortisol over-exposure. An attenuated expression of 11β-HSD2 was also found to be associated with adverse birth outcome and pregnancy related illnesses. Assessing 11β-HSD2 in saliva by applying a ratio to cortisone and cortisol levels also revealed that stressful events predict a lowered ratio. Further, a positive correlation of the ratio and neonatal birth weight and size was revealed.

4 Psychological Stress during Pregnancy

While the last chapter was dedicated to the physiological stress response during pregnancy, the following section will focus on the psychological aspects of stress and its consequences for maternal wellbeing and birth outcome parameters.

4.1 The Effects of Stress during Pregnancy

As aforementioned, stress can be categorized by their occurrence in duration, intensity and prevalence. According to Eckenrode (1984), acute stress is characterized by a short endurance, a defined initiation and stimulating in reference to specific coping mechanisms in the organism. In comparison, chronic stress is assumed to last for a prolonged period, even though the exact time remains undefined in scientific consideration (Miller, Chen, & Zhou, 2007). Persistent adverse conditions, the repeated occurrence of a stressor or the insufficient adaptation towards it can be referred to as chronic stress (McEwen, 1998). Cumulative stress exposure is associated with allostatic load and by this with health outcomes (Pearlin, Schieman, Fazio, & Meersman, 2005). Allostatic load is therefore considered to be a marker of chronic stress (Wallace & Harville, 2013). Research results differ with regard to the consequences of acute and chronic stress during pregnancy. Considering acute stressors, elevated depressive symptoms in the postpartum period were shown to be related to a more pronounced psychological stress response upon the administration of an acute stressor (TSST) during pregnancy (Nierop et al., 2006). Entringer and colleagues (2011) however, could not find weeks of gestation to be predicted by acute negative affect. The consequence in reference to chronic exposure of stress seem to be more homogeneous and will be described below.

Pregnancy is a state, which is accompanied not only by drastic physiological changes of the women, but also by socioeconomic alterations. Mainly the areas of finance, intimate relationships, obligations towards the own family, employment and pregnancy related worrying

constitute constant stressors during the time of gestation (Dunkel Schetter & Glynn, 2011). The appearance of psychological illnesses do not exceed during pregnancy in comparison to the non-pregnant state. However, the incidence of elevated maternal anxiety and depressive mood seem to be present during gestation (Ditzen & Beinder, 2011) and pregnancy related anxiety was strongly associated with preterm birth and gestational age, whereby depressive symptoms were related to low neonate birth weight (Dunkel Schetter & Tanner, 2012). Maternal chronic stress during pregnancy is assumed to influence maternal wellbeing as well as birth outcome parameters. Chronic stress, assessed at 30 weeks of gestation in 6000 participants, revealed an elevated risk for preterm delivery (Hedegaard, Henriksen, Sabroe, & Secher, 1993). Further, an increased risk (25 - 60 %) for preterm birth was found in pregnant women suffering from chronically psychosocial stress (Wadhwa, Entringer, Buss, & Lu, 2011). Wallace and Harville (2013) found increased allostatic load, assessed between 20 and 30 weeks of gestation, to predict a decrease in gestational age. In a recent review, conducted by Dunkel Schetter and Tanner (2012), it was shown, that chronic stressors, such as household strain, can be considered as predictors of preterm birth. Further, the authors postulate that chronic stressors were found to be even more robust in their effects on low birth weight. Low birth weight was also found to be predicted by the chronic stressors of unemployment and crowding and elevated the risk by 2 to 3.8 times (Borders, Grobman, Amsden, & Holl, 2007). Also, spontaneous abortion (Wainstock et al., 2013) and negative development during childhood (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003) were found to be associated with high levels of maternal anxiety and stress during pregnancy. Elevated levels of chronic stress during pregnancy is not only associated with adverse consequences for the birth outcome, but also for the maternal wellbeing. The experience of anxiety and stress during pregnancy was related to postpartum depression (Robertson, Grace, Wallington, & Stewart, 2004).

In the following, chronic stress during pregnancy and its consequences for maternal wellbeing and birth outcome parameters is described in more detail exemplary for work related stress. The recurrently daily experience of inconveniences at work can be considered as chronic stress (Bäuerlen, 2013).

4.2 Work Stress as an Example for the Chronic Psychological Stress Experience

McMichael, Spirats and Kupper introduced in 1974 the concept of the *healthy worker effect*. It is assumed that working individuals are constituted with relatively good health in order to be employed. Further, the presumed good health in workers is, according to the authors, related to lowered rates of morbidity and mortality in comparison to the general population. However, as Li and Sung (1999) illustrated in their review article, methodological issues in terms of selection and confounding biases with regard to the concept of the healthy worker effect seem to exist. Despite the methodological problems of the healthy worker effect, working during pregnancy does not represent a stressor per se. Positive factors were attributed to women, who work during their pregnancy, in comparison to non-working pregnant women, including having an education, higher levels of income, to embrace prenatal care from early pregnancy onward, and to gain adequate levels of weight during gestation (Gabbe & Turner, 1997). As Katz (2012) pointed out, pregnant women who pursue a profession, experience individually the possible burden of work related strains. When work is perceived as non-stressful, no correlations with an increased risk of maternal or pregnancy related health outcomes were shown (Bonzini, Coggon, Palmer, 2007; Katz, 2012). However, when work is perceived as stressful, associations with adverse maternal and foetal consequences were shown. These are described in detail in the following.

Work related stress can be assessed in terms of external stressors, such as long working hours or heavy lifting, or by studying internal, psychological aspects of work related stressors, for example the subjective perception of social evaluation. Work is perceived as stressful, when work related demands in terms of their quality or quantity are considered as under- or overloading. In addition, reoccurring disruptions from the work process are considered as demanding (Bäuerlen, 2013). Further, workplaces, which are characterized by external physical stressors like noise, heat or cold, air contamination by the use of chemicals, long sequences of sitting or standing, or the carriage of loads are considered to represent a source of stress for

their employees (Chamberlain, 1993). Moreover, shift-work and working at night and a long commute at peak traffic periods can constitute stress factors in relation to employment. Social conflicts with managers, colleagues or clients are also assumed to contribute to the stress experience at work (Bäuerlen, 2013). In addition, an unbalanced perception of the accomplished work and the received payment, social recognition and security of employment can constitute another form of chronic work related stress (Siegrist, 1996).

One possibility to investigate work stress represents the *job-demand-control model* (Karasek, Baker, Marxer, Ahlbom, & Theorell, 1981). Job demand includes psychological workload, working pace, and the pressure of time and tasks. Job control, on the other hand, refers to skill discretion and the autonomy of decision-making. A third dimension of the job-demand-control model is the job social support, which constitutes constructive interactions with co-workers and managers. The model assumes that work is perceived as stressful, when the job demand is experienced as high and the job control as low (Karasek, 1979). Chronic work related stress was found to be related with depressive mood, anxiety, panic attacks, sleep disturbance, hypertension, social phobia and addictive disorders (Bäuerlen, 2013; Landsbergis & Hatch, 1996; Woods et al., 2010). The wide range of adverse correlates with work related stress demonstrates the necessity to investigate associations of chronic work related stress and its predictive values for maternal wellbeing and birth outcome parameters. In the following sections, the associations of chronic work related stress and maternal wellbeing and birth outcome are described.

4.2.1 Effects of Work Stress on Birth Outcome

Investigations of work related stress during pregnancy and birth outcome revealed inconsistent results. However, it appears that particular working conditions during gestation seem to be potentially associated with adverse maternal and foetal consequences. According to the review article from Katz (2012), work related stress was found to be associated with a variety of pregnancy outcomes, including miscarriage, preterm labour and birth, reduced birth weight,

infant being small for gestational age (SGA), hypertensive disorders and preeclampsia. Occupational fatigue (this concept involves, according to Croteau, Marcoux, and Brisson (2006), a score criterion, with a scale from 0-5, for work related standing, working with industrial machines, carrying heavy loads, repeating physical tasks, working with noise, vibration, cold or chemicals and was originally developed by Mamelle, Laumon, and Lazar in 1984) was found to predict preterm birth, low birth weight, hypertension, spontaneous abortion, and foetal demise. Further, Katz (2012) showed that work, which is characterized by irregular working hours or night shift, was related to low birth weight and spontaneous abortion. Mozurkewich, Luke, Avni, and Wolf (2000) found in their review article associations of physically demanding work and preterm birth, SGA, hypertension and preeclampsia. Preterm birth was also related to a long duration of standing, shift work and night work and occupational fatigue. In contrast to Katz (2012), no association of long working hours per week and preterm birth was detected. The review article from Bonzini and colleagues (2011) showed a small but significant elevated risk of shift work predicting preterm birth, low birth weight and SGA. In a study, which examined 575 working women in every pregnancy trimester, long working hours predicted reduced foetal growth (measured by birthweight for the gestational age) at every measurement point (Hatch, Ji, Shu, & Susser, 1997). However, no associations were found for work related standing, heavy lifting, or climbing in this study. Preterm birth was found to significantly correlate with long working hours (> 42h) and long periods of standing (> 6h) in a study investigating work stress during pregnancy in European countries (Saurel-Cubizolles et al., 2004).

Additionally, researchers found that specific types of employments can be associated with an elevated risk for adverse birth outcome. These occupational categories were characterized by being physically demanding, but results appear to be not fully homogeneous. Pinhas-Hamiel and colleagues (1999) investigated 207 pregnant women working as physicians or being still in residency in Israel. The authors observed a significant difference in their study population and the general population with regard to the rates of stillbirth and preterm delivery, with the study population distributing elevated risks. It was concluded, that the stressful working environment

and high pressure in a hospital might increase the risk of complicated pregnancies and adverse birth outcomes. However, being in medical residency was not associated with an elevated risk for preterm delivery, low birth weight or spontaneous abortion in the review article from Gabbe and Turner (1997). McDonald and colleagues (1988) reported elevated levels of low birth weight and preterm delivery for pregnant women being employed in service and manufacturing sectors. Rates of preterm birth were even more elevated in women working in food and beverage sectors or as psychiatry nurses. These elevations also appeared for low birth weight for women in the service industry, cleaners and for women working in metal and electrical industries.

Psychological aspects in comparison to external work related stressors seem to be studied to a far lesser extent. The focus in research was mainly put on external work related strains. However, psychological work related stress was associated with an elevated chance of delivering a neonate with low birth weight, having a miscarriage, preterm labour and birth and hypertension (Katz, 2012). Another example, where the subjective perception of work stress during pregnancy is taken into account, is the study conducted by Vrijkotte, van der Wal, van Eijsden, and Bonsel (2009). Psychological work related stress in terms of job demand and job control and external work related stress in terms of working hours per week, were assessed in 7135 pregnant women in early pregnancy (mean weeks of gestation: 13) and their relation to the birth outcome variables of low birth weight and SGA. A significant reduction in birth weight was shown to be associated with long working hours per week (> 32h per week) and high job strain. Further, the authors found that high job strain predicted SGA. The combination of high job strain and long working hours showed the largest effects on a reduced birth weight and the risk for SGA (Vrijkotte et al., 2009). Saurel-Cubizolles and collegues (2004) also found that women, who were dissatisfied with their work, experienced an elevated risk for preterm birth. Additionally, the authors reported that heteronomy was associated with preterm birth in specific European countries (France, Germany, Italy, Slovenia, the Czech Republic, Finland, Greece, Ireland, Scottland, Spain, Sweden, and the Netherlands). Contradictory, the effects of working in a high strain job during pregnancy with regard to preterm delivery were not found by Brett,

Strogatz and Savitz (1997). The authors investigated 421 participants six weeks postpartum. Job strain was assessed retrospectively for the entire pregnancy and for the third trimester. A small elevation in the risk of delivering a preterm neonate was detected in the high job strain group, in comparison to the low job strain group. However, this increase was of non-significance at every measurement point. Albeit, the authors found pregnant women, who worked more than 30 weeks during gestation or full time at a high strain job, to have a moderate elevated risk for preterm delivery.

4.2.2 Effects of Work Stress on Maternal Wellbeing

The influence of work related stress during pregnancy on maternal wellbeing appears to be strongly understudied. Only very few work groups investigated these effects over the course of gestation. In general, research has displayed consistency in the associations between work related strains and psychological stress (Lopes, Araya, Werneck, Chor, & Faerstein, 2010; Van der Doef & Maes, 1999). In a recent study, Sanguanklin and colleagues (2014) investigated 300 pregnant women (weeks of gestation: 26-38) with regard to their psychological distress, evoked by job strain. Job strain was assessed in terms of the psychological perception of job demand and control. Women who scored high in demand and low in control were considered as burdened by the authors. Job strain was found to be positively associated with psychological distress (Sanguanklin et al., 2014). However, there are also studies reporting a possible protective effect of working during and after pregnancy, as outlined in the following.

Adejumo (2008) examined the associations of work overload and psychological wellbeing in 200 career women (holding positions in banking, engineering, nursing, teaching and medicine) during the second and third trimester of pregnancy. The author found, that women scoring high in work overload, revealed elevated psychological wellbeing. These results were not expected and the author explained it by postulating that challenges in career expectations may be seen as motivating and by this leading to elevated wellbeing. Further, a recent study from Lewis and colleagues (2017) investigated the association of employment

status and depressive symptoms in 124 women at seven months postpartum. The authors reported that 84% of the study population had experienced depressive symptoms in the past. It was found that women who pursued a profession seven months after delivery actually reported lower depressive symptoms in comparison to non-working women. This finding proved also significance for women with a depressive history. The authors concluded that working at seven months postpartum seems to have a protective effect for the development of a postpartum depression. In addition, Lewis and colleagues (2017) also discussed that the protective effect of employment postpartum might be constrained by factors such as total workload or psychological demands. This supports the earlier argument that working during or after pregnancy is not per se a risk factor- it might even has a protective effect. However, when work related aspects are perceived as stressful, maternal wellbeing might be compromised.

4.3 Summary of the Psychological Findings during Pregnancy

During the time of gestation, especially chronic maternal stress seems to constitute a state, which can be associated with adverse consequences for the expecting mother and the foetus. Allostatic load is assumed to symbolize a marker for chronic stress and reveals different health consequences on a physiological, as well as psychological level. Pregnancy, characterized by change and adaptation, can represent a time of a potentially elevated risk for maternal chronic stress. Maternal chronic stress was associated with birth outcome parameters like preterm delivery or low birth weight, and with regard to the expectant mother with postpartum depression. As an example for chronic stress, work related stress during pregnancy was analysed. It was found that, when work does not represent a source of stress, no associations with adverse maternal or foetal health outcomes were reported. However, when work contributes to the quotidian stress experience and amounts by this to chronic stress, associations were found. Researchers mostly investigated work stress either by analysing external factors, such as long working hours, or by using the job-demand-control model. When the demands

were considered as high and the control as low, the experience of stress resulted. The examination of maternal work stress during pregnancy with regard to foetal health consequences revealed heterogeneous results. In respect to external work related factors, occupational fatigue, shift or night work, long duration of standing, and long working hours were found to inconsistently reveal associations with birth outcome parameters. Further, physically demanding occupational categories, like being employed as a physician or working in the service sector during pregnancy, seem to be related with adverse birth outcome. In addition, but to a much lesser extent studied, psychological factors of work related stress proved significant associations with adverse birth outcome parameters. Again, these findings displayed inconsistencies. The effects of work stress on maternal wellbeing seem understudied and results are again not consistent. However, this might be attributed to methodological differences in assessing chronic maternal work stress and to the diverse study populations in socioeconomic terms.

5 Summary, Conclusions and Research Questions

To date the underlying psychoendocrine mechanisms in the human stress response are not fully understood. The concept of stress is characterized by a long history with steady adaptions and new scientific approaches. Research investigated the stress response in terms of psychological and biological aspects. It was found that psychological as well as external stressors can elicit a psychobiological stress experience. Chronic stressors are assumed to predict negative health consequences and are studied mainly in relation to the stress hormone cortisol. During the time of human gestation, the psychological, as well as the endocrine stress response, elicited great interest in various research disciplines. It was shown, that maternal stress during pregnancy can have adverse consequences for the maternal wellbeing and the unborn child. Research appears to be heterogeneous though, in terms of outcomes and methodical conduction.

The maternal and foetal stress physiology was intensively studied and an over-exposure of maternal stress hormones was associated with adverse consequences. The protective mechanism of 11β HSD2 was shown to potentially preserve the unborn child from maternal stress. How these mechanisms exactly work and if they differ with regard to the three pregnancy trimesters remains partly elusive. Further, it is not fully understood how different categories of stress during pregnancy, here investigated exemplary for chronic work related stress, predict birth outcome parameters and maternal wellbeing, as research results were inconsistent.

Therefore, the aim of the current work was to investigate how specific aspects of chronic stress during pregnancy can be associated with maternal wellbeing and birth outcome parameters and to explore how the stress hormones cortisone, cortisol and its ratio, as a marker for the enzyme 11β HSD2, develop over the course of gestation. Two empirical studies with longitudinal designs were conducted to elaborate these questions. As presented in the theoretical background, maternal chronic work stress revealed heterogeneous results with regard to birth outcome and was mainly studied by considering external work related strains,

like long working hours. Psychological aspects were of less interest in the literature. Further, the focus was put on birth outcome parameters and maternal wellbeing was almost unregarded. Similarly, the existence of studies investigating cortisol and cortisone over the entire course of gestation and postpartum appears very limited and examinations of 11β HSD2 over the entire course of pregnancy seem non-existent. However, as psychological aspects of chronic work stress can potentially elicit adverse consequences for maternal wellbeing and birth outcome, it appears critical to investigate these aspects. Further, the exploration of the development of maternal cortisol and cortisone over the course of gestation in relation to the protective mechanisms of the enzyme 11β HSD2 is, in consideration of the very limited research, indicated.

In the first empirical study, we investigated the association between five different psychological aspects of chronic work related stress and maternal wellbeing and birth outcome parameters in 100 healthy pregnant women. Further, two subsamples, namely women with high job strain and women with low income, were considered in the analyses. Previous research results demonstrated a negative correlation between psychological chronic stress and maternal wellbeing and birth outcome. Therefore, we expected that the examined aspects of work stress reveal negative associations with maternal wellbeing and birth outcome. Further, we presumed a more pronounced negative association between the correlates within the subsamples.

In the second study, we examined cortisol and cortisone, assessed in saliva, over the course of gestation in four-week intervals. Further, the ratio of salivary cortisone and cortisol was investigated, as it is assumed in current research, that the ratio is a representative biomarker for the salivary 11β HSD2 activity. The examination of cortisone, cortisol and their ratio, assessed in saliva samples, is mainly concentrated on later stages of pregnancy (second and third trimester) and often explores only punctually the development by two or three measurement points. Due to one study (Allolio et al., 1990), which demonstrated that cortisol levels in saliva increases from gestational week 25 onwards, we expected an increase in cortisol with progressing gestation, including a simultaneous increase in the ratio of cortisone and cortisol.

In sum, the aim of this thesis was to provide new insights about specific factors of chronic work stress during pregnancy, which can predict maternal wellbeing and birth outcome parameters, as well as the exploration of the development of the hormones cortisone, cortisol and their ratio over the course of gestation.

PART II:
EMPIRICAL STUDIES

6 Does Psychological Work Stress Impact Maternal Wellbeing or Neonatal Birth Outcome?

6.1 Introduction

An excessive experience of maternal stress during pregnancy accompanied by adverse mental wellbeing is associated with unfavourable perinatal outcomes (Bonzini et al., 2007; Cardwell, 2013; Ghaemmaghami et al., 2014; Ghaemmaghami & Ehlert, 2015; Glover, 2014; Sanguanklin et al., 2014).

One factor which can contribute to the experience of stress during pregnancy is work stress. In Switzerland, one third of the working population experiences chronic work-related stress (Grebner, Berlowitz, Alvarado & Cassina, 2011). Healthy pregnant women work until the expected date of delivery, since maternity leave in Switzerland only begins at the child`s birth (Systematische Rechtssammlung des Bundesrechts, 2016). Accordingly, also pregnant women might be at risk of experiencing stress at work. Work stress can be examined by considering either external factors, such as long working hours, or internal factors, such as the subjective psychological perception of work stress. External factors have proven associations with impacted birth outcome. Yet, less is known with regard to maternal wellbeing, as well as the psychological perception of stress, as the focus of work stress research in pregnancy prevalently refers to external factors.

Studies examining external work stress and birth outcome showed that regular work-related standing up during late pregnancy is associated with the delivery of a small-for-gestational-age neonate, and night work is associated with preterm labour (Fortier, Marcoux, & Brisson, 1995). Moreover, long working hours in late pregnancy seem to have a negative effect on foetal growth and these effects are elevated when pregnant women are additionally exposed

to work-related climbing, heavy lifting or prolonged standing (Hatch et al., 1997). Additionally, long working hours seem to increase the risk of preterm delivery (Luke et al., 1995), and occupational fatigue (prolonged standing, working on industrial machines, carrying heavy loads, physical tasks, and working with noise, chemicals, vibration or cold) has been associated with preterm birth (Croteau, Marcoux, & Brisson, 2007). Also, shift work exerts a small but significant effect on birth outcome with regard to preterm delivery, low birthweight and small-for-gestational-age (Bonzini et al., 2011).

However, as stress is generated by the individual perception of demands (Lazarus & Folkman, 1984), external work-related strains may not be perceived by each individual as stressful. The perception of psychological work-related factors may contribute to stress development, and should therefore be considered when examining the association between work stress and maternal and foetal outcomes.

Compared to external stressors, psychological work stress during pregnancy and its consequences for maternal wellbeing and birth outcome have received less research attention. High job strain has been found to contribute to psychological distress in full-time employed pregnant women (Sanguanklin et al., 2014). Moreover, pregnant women with passive jobs (i.e. low demand and low control) seem to be at greater risk to deliver children with lower birthweight (Lee et al., 2011). Furthermore, high job strain in early pregnancy has been associated with delivering a small-for-gestational-age neonate (Vrijkotte et al., 2009).

Another study suggested that associations differed depending on occupational categories, with managers and professionals being less likely to experience preterm delivery than skilled (precision production workers) and unskilled (operators/- fabricators) workers (Gabbe & Turner, 1997). An increased risk of low birthweight and preterm birth, among pregnant women working in service or manufacturing was postulated additionally (McDonald et al., 1988). These findings indicate, that work stress can elevate a pregnant woman`s overall stress level, and that certain groups of working pregnant women can be characterized according to their risk of adverse birth outcome.

To summarize, work can represent a source of stress, and as a consequence, it can have detrimental effects on maternal wellbeing and birth outcome. To date, research on work stress has mainly concentrated on external factors, while less attention has been paid to psychological work-related factors. Moreover, regarding the consequences of work stress, most studies have focused on the foetus, while maternal wellbeing has received less attention. Therefore, the aim of the present study was to explore different aspects of psychological work stress during pregnancy and its consequences for maternal wellbeing and birth outcome.

Based on previous research, we assumed that maternal wellbeing during pregnancy will decrease with increasing psychological work stress; adverse birth outcome will be associated with elevated psychological work stress levels; and lower maternal wellbeing and adverse birth outcome will occur more often in in economically and work-related disadvantaged populations.

6.2 Methods

6.2.1 Participants and Procedure

This study was implemented within a longitudinal assessment of the experience of stress during pregnancy. The study protocol was approved by the local Ethics Committee and conducted in cooperation with the University Hospital Zurich from 2013 to 2016. Exclusion criteria were maternal age under 18 or over 45 years, artificial fertilization, multiple gestation, maternal or foetal medical or psychological conditions, maternal over-/underweight, current use of medication or psychotropic substances, current alcohol or tobacco consumption, and a protein-restricted diet. In sum 284 pregnant women were interested in participating in the study, of whom 116 pregnant women met the inclusion criteria. Sixteen of these participants were excluded due to pregnancy loss or dropout; thus a total of 100 women were examined. Participants provided informed consent. Women in early pregnancy (week of gestation: M=9.59, SD=2.15) were invited to the Department of Clinical Psychology and Psychotherapy at the University of Zurich, where work stress and maternal wellbeing was assessed. Work-

related data were surveyed again in the third trimester, while maternal wellbeing was additionally assessed three weeks postpartum.

All participants were in employment, except for seven, who reported to be housewives (six of whom had at least one child under the age of seven years). We included housewives in the analysis as household labour is comparable to paid labour in terms of workload. In Switzerland, for women with at least one child under the age of seven, the average number of working hours per week (seven days) amounts to 55.5 hours of household labour (Bundesamt für Statistik, 2014). Moreover, a woman`s wellbeing seems to be positively affected when household labour is valued with equal appreciation to paid labour (Lee & Waite, 2010).

6.2.2 Outcome Measures

Psychological Measures

Maternal work stress was assessed with the Trier Inventory of Chronic Stress (TICS), which measures chronic exposure to stress within the past three months (Schulz & Schlotz, 1999). The TICS is a standardized questionnaire, which examines nine aspects of chronic stress (Schulz, Schlotz, & Becker, 2004). The following work-related subscales and the *screening scale of chronic stress* of the TICS were taken into account for the analyses: *work overload*, *pressure to perform*, *work disconnect*, *excessive demands at work*, and *lack of social recognition*. The TICS was completed by the total study group in early pregnancy (TICS T1, N=100, week of gestation: M=9.59, SD=2.15) and in the third trimester (TICS T2, N=88, week of gestation: 33). Cronbach's alpha has been reported to be good to excellent (α=.84 to .91). In the current analysis, Cronbach's alpha demonstrated good reliability at T1 (α=.83) and at T2 (α=.86). This study used the original German version of the TICS (Schulz et al., 2004). Sum scores of the separate subscales served as independent variables.

The validated German version of the Edinburgh Postnatal Depression Scale (EPDS) was applied in early pregnancy to assess maternal wellbeing (EPDS T1, N=100, weeks of gestation:

M=9.59, *SD*=2.15), (Bergant, Nguyen, Heim, Ulmer & Dapunt, 1998; Cox, Holden, & Sagovsky, 1987) and three weeks postpartum (EPDS postpartum). The EPDS is a screening instrument for postnatal depression and can be applied during pregnancy as well (Cox et al., 1987; Murray & Cox, 1990). Cronbach's alpha was reported to be good (α=.87) (Cox et al., 1987). In the current sample, Cronbach's alpha can also be considered as good at T1 (α=.79) and postpartum (α=.78). The sum score served as dependent variable.

Birth Outcome Measures

Data regarding neonatal birth outcome were obtained from medical records. Week of gestation at birth, size of the newborn at birth and birthweight were used as separate dependent variables.

6.2.3 Data Analysis

Data were analysed using IBM SPSS Statistics, version 22. Prior to statistical analysis, normal distribution was assessed. Since the TICS and EPDS were non-normally distributed, non-parametric tests and the bootstrap method were applied (Field, 2009). A two-step procedure was used to determine the predictive value of work stress for maternal wellbeing and birth outcome in order the to prevent multiple testing (Ghaemmaghami et al., 2014). As a first step, Spearmen correlations were calculated to examine the relationship between work stress, maternal wellbeing and birth outcome. The second step included separate bootstrap regression analyses with those work-related stress variables that were significantly associated with maternal wellbeing and birth outcome. This procedure was chosen due to high correlations among the TICS subscales. Week of gestation at birth, neonatal size and birthweight served as separate dependent variables. Before the main analysis, control variables were determined based on bivariate correlations between maternal characteristics and work stress. These included educational level (correlated with work overload T1; *r*=.20, *p*=.04, pressure to perform T1; *r*=.26, *p*=.01; work overload T2; *r*=.23, *p*=.03; pressure to perform T2; *r*=.29, *p*=.01),

workload (correlated with pressure to perform T1; r=.25, p=.01; work overload T2; r=.28, p=.01; pressure to perform T2; r=.25, p=.02), income (correlated with pressure to perform T1; r=.24, p=.02), job strain (correlated with work overload T1; r=.23, p=.04, pressure to perform T1; r=.25, p=.02; work overload T2; r=.23, p=.05) and marital status (correlated with pressure to perform T2; r=.25, p=.02; lack of social recognition T2; r=.40, p=.00; screening scale of chronic stress T2; r=.27, p=.01). Control variables for birth outcome measures included marital status (correlated with size at birth; r=-.29, p=.01) and week of gestation at birth (correlated with birth size; r=.568, p=.00 and weight; r=.531, p=.00). Data analyses were conducted with the total study group (total sample, N=100). Additionally, subsamples were created for participants reporting medium to high job strain (elevated job strain, N=35), and participants with an annual taxable income under 40`000.- CHF (low income, N=23). To consider changes over time, the analyses conducted for T2 and postpartum contained those parameters assessed at T1 as control variables. All analyses were one-tailed due to the directional hypotheses, and the level of significance was set at p<.05. Participants reported higher depression values at T1 (M=5.73, SE=.46) than postpartum (M=4.00, SE=.38), t (77)=-3.21, p<.002, r=.34. There were no significant changes in the separate work stress subscales from T1 to T2.

6.3 Results

6.3.1 Sample Characteristics

Participants` age ranged from 23 to 42 years (M=31.25, SD=3.89). The majority was of Swiss (75%) or German (21%) nationality. At T1, 55% of the participants were married and 44% stated to be cohabitating with the father of the child. In sum, 43% of the participants had a university degree and 32% had high school-leaving qualifications. Concerning workload, 43% of the participants reported working more than 34 hours a week, 37% worked 17 to 33 hours per week, 13% worked between four and 16 hours per week and seven percent of the participants stated that they were housewives. Regarding work stress, 58% reported no or minor

burden and 42% reported a medium, fairly high or high burden. 50% of the participants reported earning a taxable annual income of more than 60`000.- CHF, 26% between 40`000.- and 60`000.- CHF and 24% less than 40`000.- CHF. The pregnancy characteristics and birth outcome measures are presented in Table 1. Parity is divided into primipara, referring to women with no previous pregnancy and multipara, referring to women with previous pregnancies.

Table 1: Pregnancy characteristics of the study population at T1 and birth outcome measures.

Characteristics	*N*	%	*M (SD)*	Range
Week of gestation at T1	100		9.59 (2.15)	5- 16.29
Pregnancy intention				
Planned	88	88		
Unplanned	12	12		
Parity	100			
Primipara	62	62		
Multipara	38	38		
Previous pregnancy loss	100			
No				
Yes	89	89		
	11	11		
Pregnancy week at delivery				
< 37	72			34-41
37-40	4	5.6		
> 40	65	90.2		
	3	4.2		
Size of the child (cm)	72		49.85 (2.25)	43-56
< 50	29	40.3		
>50	43	59.7		
Weight of the child (gr)	72		3288.71 (502.59)	2120-4540

6.3.2 The Predictive Value of Work-Related Stress at T1 for EPDS Scores at T1

As shown in Tables 2 and 3, work stress at T1 predicted decreased EPDS scores in all study groups. The results indicated that all work-related subscales of the TICS were significantly associated with decreased EPDS scores in the total study group. Only *pressure to perform* was

of borderline significance. Within the group with elevated job strain, *lack of social recognition* and the *screening scale of chronic stress* were found to be significantly associated with decreased EPDS scores. Regarding the low-income group, all work-related subscales predicted lower EPDS scores, except for *excessive demands at work* and *work overload*, which reached borderline significance.

Table 2: Summary of the separate bootstrap regression analyses for work stress at T1 predicting EPDS scores at T1 in the total study group.

	Maternal Wellbeing				
T1	Total sample				
Variables	*B*	*SE B*	*p*	*Adjusted* R^2	R^2 *change*
Work overload	.137	.073	.034	.162	.041
Pressure to perform	.092	.069	.097	.138	.022
Work disconnect	.296	.087	.001	.243	.121
Excessive demands at work	.373	.143	.008	.213	.091
Lack of social re-cognition	.490	.122	.001	.259	.134
Screening scale of chronic stress	.287	.047	.001	.389	.261

Control variables: Marital status, educational level, work-load, income, job strain
*p<.05, **p<.01, ***p<.001

Table 3: Summary of the separate bootstrap regression analyses for work stress at T1 predicting EPDS scores at T1 in the analysed subsamples.

	Maternal wellbeing									
T1	Elevated job strain					Low income				
Variables	*B*	*SE B*	*p*	*Adjusted* R^2	R^2 *change*	*B*	*SEB*	*p*	*Adjusted* R^2	R^2 *change*
Work overload	.173	.133	.093	.122	.075	.217	.216	.079	.035	.094
Pressure to perform	.137	.140	.158	.079	.040	.338	.143	.010	.217	.285
Work disconnect	.172	.152	.142	.079	.041	.363	.281	.049	.038	.149
Excessive demands at work	.228	.210	.126	.085	.046	.351	.298	.133	.039	.090
Lack of social recognition	.457	.232	.029	.338	.238	.778	.286	.007	.260	.319
Screening scale of chronic stress	.241	.094	.010	.280	.209	.368	.114	.009	.318	.362

Control variables: Marital status, educational level, work-load, income, job strain
*p<.05, **p<.01, ***p<.001

6.3.3 The Predictive Value of Work-Related Stress at T1 for EPDS Scores Postpartum

In the total study group, *work disconnect* at T1 predicted lower EPDS scores postpartum (*B*=.189, *SE B*=.092, *p*=.022, adjusted R^2=.064, R^2change=.068). A similar trend appeared for the *screening scale of chronic stress* (*B*=.111, *SE B*=.071, *p*=.067, adjusted R^2=.054, R^2change=.058). Within the groups with elevated job strain and low income, none of the work-related subscales reached significance.

6.3.4 The Predictive Value of Work-Related Stress at T2 for EPDS Scores Postpartum

In the total study group, *work disconnect* (*B*=.372, *SE B*=.214, *p*=.05, adjusted R^2=.148, R^2change=.105) and the *screening scale of chronic stress* (*B*=.094, *SE B*=.105, *p*=.05, adjusted R^2=.020, R^2change=.022) reached statistical significance. *Excessive demands at work* was of borderline significance (*B*=.267, *SE B*=.170, *p*=.062, adjusted R^2=.058, R^2change=.051). Within the groups with elevated job strain and low income, none of the work-related subscales proved significance (*p*>.10 for all variables).

6.3.5 The Predictive Value of Work-Related Stress at T1 for Neonatal Birth Outcome

Week of gestation at birth

Pressure to perform predicted week of gestation at birth in the total study group (*B*=-.053, *SE B*=.025, *p*=.023, adjusted R^2=.037, R^2change=.078) and in the group with elevated job strain (*B*=-.061, *SE B*=.038, *p*=.05, adjusted R^2=.099, R^2change=.087). For the low-income group,

lack of social recognition predicted a decreased number of pregnancy weeks (*B*=-.170, *SE B*=.116, *p*=.05, adjusted R^2=.180, R^2change=.308).

Size at birth

In the elevated job strain group, a smaller size at birth was predicted by the *screening scale of chronic stress* at borderline significance (*B*=-.08, *SE B*=.045, *p*=.051, adjusted R^2=.109, R^2change=.085).

Birthweight

In the elevated job strain group, *pressure to perform* (*B*=-25.37, *SE B*=15.373, *p*=.047, adjusted R^2=.034, R^2change=.106) and *excessive demands at work* (*B*=-45.469, *SE B*=18.490, *p*=.009, adjusted R^2=.076, R^2change=.143) predicted birthweight. Furthermore, the *screening scale of chronic stress* (*B*=-23.313, *SE B*=11.191, *p*=.02, adjusted R^2=.061, R^2change=.129) was significantly associated with birthweight.

6.3.6 The Predictive Value of Work-Related Stress at T2 for Neonatal Birth Outcome

Week of gestation at birth

In the total study group, *pressure to perform* (*B*=-.063, *SE B*=.034, *p*=.038, adjusted R^2=.051, R^2change=.096) and *lack of social recognition* (*B*=-.136, *SE B*=.078, *p*=.05, adjusted R^2=.043, R^2change=.089) predicted week of gestation at birth.

Size at birth

In the elevated job strain group, *lack of social recognition* (*B*=-.315, *SE B*=.152, *p*=.029, adjusted R^2=.163, R^2change=.219) and *excessive demands at work* (*B*=-.177, *SE B*=.096, *p*=.039, adjusted R^2=.099, R^2change=.163) predicted size at birth. Further, *pressure to perform* (*B*=-.166, *SE B*=.060, *p*=.008, adjusted R^2=.260, R^2change=.303) and the *screening scale of chronic stress* predicted size at birth (*B*=-.116, *SE B*=.056, *p*=.023, adjusted R^2=.145, R^2change=.203).

Birthweight

In the total study group, the *screening scale of chronic stress* reached borderline significance (*B*=-13.211, *SE B*=7.865, *p*=.055, adjusted R^2=.072, R^2change=.038). In the elevated job strain group, the *screening scale of chronic stress* (*B*=-25.92, *SE B*=11.627, *p*=.015, adjusted R^2=.092, R^2change=.206) and *pressure to perform* (*B*=-35.233, *SE B*=14.500, *p*=.008, adjusted R^2=.171, R^2change=.275) predicted birth weight. In the low-income group, by *lack of social recognition* at borderline significance (*B*=-102.610, *SE B*=55.409, *p*=.051, adjusted R^2=.296, R^2change=.409) predicted birthweight. The results for work stress in the third trimester of pregnancy as a predictor of birth outcome no longer proved to be significant when the magnitude of change over time was taken into account, and cross-sectional results are presented within the section of "the predictive value of work related stress at T2 for neonatal birth outcome".

6.4 Discussion

This study investigated associations of psychological work stress with maternal wellbeing and neonatal birth outcome in the total study group as well as in women with elevated job strain and low income.

First, the main significant contribution lies in showing associations of diverse aspects of psychological work stress with lower maternal wellbeing in early pregnancy in all study groups. Concerning the association between work stress in early pregnancy and decreased maternal wellbeing postpartum, significant associations were found in the total study group, but not within the subsamples. The same results occurred for work stress in the third trimester and maternal wellbeing postpartum. Considering recent literature, an effect of work overload on women`s psychological wellbeing during the second and third trimester was reported (Adejumo, 2008). While the present study did find *work overload* in early pregnancy to be significantly associated with maternal wellbeing during that phase of their pregnancy, this association was not found for the third trimester in the total study group. The current findings showed a more homogeneous outcome for all groups in early pregnancy compared to late pregnancy and postpartum. Fewer subscales were associated with maternal wellbeing in the third trimester and at postpartum. This might be explained by the fact that pregnancy involves a process of adaptation: A failure to sufficiently accomplish this adaption may lead to elevated stress, which might be amplified by work stress (Pattison & Gross, 1996). Women in early pregnancy may not have had sufficient time to fully adapt to their new role as expectant mother. This might explain why they are more likely to experience decreased wellbeing when additionally faced with psychological work stress compared to at the end of their pregnancy. In general, our results seem consistent with recent literature, where elevated psychological distress among pregnant women experiencing psychological work stress was reported (Sanguanklin et al., 2014). In the current analysis, the two aspects of stress *work disconnect* and *lack of social recognition* appear to be robust and most frequently predicted maternal wellbeing when taking all study groups and time points into account. Considering the outcomes regarding the *screening scale of chronic stress* and its association with lower maternal wellbeing, this can be embedded into the literature, as chronic stress has been found to predict depressive mood (Brummelte & Galea, 2010; Ehlert, 2004).

Second, aspects of psychological work stress at different time points during pregnancy were associated with adverse birth outcome in all study groups. Psychological work stress in

early pregnancy predicted week of gestation at birth in all study groups, and predicted size at birth and birthweight in the elevated job strain group. In the third trimester of pregnancy, work stress was associated with week of gestation at birth in the total study group, size at birth in elevated job strain group and birthweight within all groups. With regard to the outcomes in early pregnancy, the results of the current study correspond to the recent literature. For instance, women at 20 or more weeks of gestation, holding passive jobs (low demand and low control) or experiencing high job strain delivered children with lower birthweight and reduced gestational age at birth (Lee et al., 2011). It was also found that women with mental work stress were more likely to experience preterm birth (Mamelle & Munoz, 1987). These findings were confirmed by another study, which investigated low birthweight and small-for-gestational-age as a consequence of work stress in early pregnancy (Vrijkotte et al., 2009). The authors found effects of high job strain on lower birthweight and of moderate to high job strain on an increased risk of small-for-gestational-age.

Taking occupational and socioeconomic factors in the current study into account, psychological work stress during pregnancy seems to be associated not only with maternal wellbeing and the birth outcome measures week of gestation at birth and birthweight, but additionally with size at birth. The elevated job strain group showed the most frequent associations with birth outcome parameters. This supports the assumption of risk groups among pregnant women, who seem more likely to experience decreased wellbeing and impaired birth outcome as a consequence of psychological work stress. So far, adverse birth outcomes have been reported to be associated with work-related maternal stress in late pregnancy (Fortier et al., 1995; Hatch et al., 1997; Naeye & Peters, 1982). This study shows that also early pregnancy should be considered. The present work makes an innovative contribution, by investigating diverse aspects of psychological work stress in early and late pregnancy within the total study group and two subsamples with regard to maternal wellbeing in early pregnancy and postpartum as well as birth outcome.

6.4.1 Limitations, Strengths and Future Studies

The pregnant women in this study were well-educated, healthy, had a relatively high income, and exhibited no risk behaviour, such as smoking, or medical conditions. These factors might limit the generalizability of the current findings, but at the same time, might also have led to an underestimation of the associations of work stress with maternal wellbeing and birth outcome. Future studies should consider investigating pregnant women with more diverse socioeconomic backgrounds. Data on psychological work stress and maternal wellbeing were self-reported. Future research should include both self-assessment and objective work-related data, like shift work, to achieve an even broader overview of which aspects of work stress correlate with maternal wellbeing and birth outcome.

Despite the aforementioned limitations, this study contributes to and extends the current research on psychological work stress during pregnancy. One of strengths of the present work is that psychological work stress was investigated by considering five different aspects. Since stress is defined as a subjective and individual approach towards different situations (Lazarus & Folkman, 1984), objective work-related factors may not automatically represent stress for every individual. The assumption that work can only be associated with adverse birth outcome when it is perceived as stressful is supported by several findings (Bonzini et al., 2007; Katz, 2012; Mozurkewich et al., 2000). Furthermore, several time points were included in the analysis. This allowed detailed determination of when aspects of psychological work stress are associated with maternal wellbeing and birth outcome.

6.4.2 Conclusion

If work is perceived as stressful, negative effects for maternal wellbeing and neonatal birth outcome can result. Especially the subscales *work disconnect* and *lack of social recognition* predicted maternal wellbeing. Psychological work-related stress should be considered particularly in early pregnancy to predict neonatal birth outcome. Therefore, pregnant women

should be individually assessed regarding their stress experience, and work situations might be adapted to prevent adverse consequences.

7 Maternal Salivary Cortisol, Cortisone and their Ratio Over the Course of Human Gestation

7.1 Introduction

Over-exposure of the foetus to glucocorticoids has been linked with unfavorable development and health outcomes in later life (Duthie & Reynolds, 2013). A determining role is attributed to cortisol, which is released as the end-product of the hypothalamic-pituitary-adrenal- (HPA) axis and characterized by a circadian rhythm (Entringer et al., 2011). Cortisol is a prominent biological stress response marker, which can be assessed in saliva, where it represents the unbound, bioactive form (Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007). Over the course of pregnancy, basal levels of salivary cortisol rise constantly from 25 weeks` gestation, and attain twice as high levels in the third trimester compared to the non-pregnant state (Allolio et all., 1990). The cortisol awakening response (CAR) is used to assess the naturally occurring stress response and describes the dynamics of cortisol secretion after morning awakening (Stalder et al., 2016). Stress and psychological wellbeing seem to be related to the magnitude of the CAR (Steptoe et al., 2003). The CAR is defined by a 50-75% increase in free cortisol levels upon awakening, reaching peak secretion approximately 30 minutes later, followed by a constant decrease over the day, displaying lowest levels during the night (Pruessner et al., 1997). The CAR seems to be preserved in third trimester pregnant women (de Weerth & Buitelaar, 2005). Research suggests that the CAR becomes more attenuated with advancing gestation (Entringer et al., 2010; La Marca-Ghaemmaghami & Ehlert, 2015). However, genuine longitudinal studies are still limited (Allolio et al., 1990; Douglas, 2010) and there are hardly any publications on the development of the CAR from very early pregnancy to the postpartum period.

The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol into its inert metabolite cortisone (Marsit, Maccani, Padbury, & Lester, 2012; Murphy, Clark, Donald, Pinsky, & Vedady, 1974). This enzyme is located in various body tissues, like the parotid glands (Edwards et al., 1988; Perogamvros et al., 2009; Smith et al., 1996). Its activity affects the concentrations of cortisol in the saliva (Perogamvros, Keevil, Ray, & Trainer, 2010; Perogamvros, Ray, & Trainer, 2012). Indeed, salivary cortisol only represents 50-60% of plasma cortisol levels due to 11β-HSD2 (Meulenberg, Ross, Swinkels, & Benraad, 1987). Findings also suggest that due to 11β-HSD2, salivary cortisone may reflect free blood cortisol levels more accurately than salivary cortisol does (Meulenberg et al., 1987). However, the enzyme activity has largely been overlooked in stress research (Gröschl, 2008; Perogamvros et al., 2012).

The cortisone-cortisol ratio has been used as a proxy to measure salivary 11β-HSD2 activity (Ghaemmaghami et al., 2014; Perogamvros et al., 2009). It is assumed that the 11β-HSD2 expression in the parotid glands rises over the course of pregnancy, as higher salivary cortisone than cortisol levels were found (Meulenberg & Hofman, 1990). By this metabolism, the potential negative effects of cortisol might be dampened, although the specific underlying mechanisms are not fully understood yet (La Marca-Ghaemmaghami et al., 2013). Further, the activity of 11β-HSD2 in the parotid glands seems to be affected by maternal stress. Our research group found that the activity of maternal salivary 11β-HSD2 is transiently decreased in response to an acute stressor in the second trimester of pregnancy (Ghaemmaghami et al., 2014). Further, a positive association between emotional support during pregnancy and the cortisone-cortisol ratio in saliva, which indicates elevated salivary 11β-HSD2 activity, was reported (La Marca-Ghaemmaghami et al., 2013). The 11β-HSD2 activity in the parotid glands may also reflect a protective mechanism against maternal glucocorticoid over-exposure to the foetus. It was found that the cortisone-cortisol ratio predicted higher birth-weight and size at birth (Ghaemmaghami et al., 2014). Another study found the maternal bedtime salivary cortisone-cortisol ratio to be inversely associated with birth-weight (Wilson & Thayer, 2016). To date, the course of salivary 11β-HSD2 across gestation remains understudied. The study at hand aims at exploring how the

morning awakening response of cortisol, cortisone and their ratio develops over the course of gestation, beginning from early pregnancy and reaching into the early postpartum period.

7.2 Subjects and Methods

7.2.1 Participants

This study was conducted from 2013 to 2016 and approved by the Ethics Committee of the Canton of Zurich, Switzerland. The project was performed in cooperation with the University Hospital Zurich. A total of 284 pregnant women were interested in participating, of which 116 women met the inclusion criteria. Due to pregnancy loss or study termination, 16 of these women were excluded. The final sample size included 100 participants. All participants provided informed consent. Exclusion criteria were maternal age under 18 or above 45 years, artificial fertilization, multiple gestation, maternal or foetal complications, current maternal psychiatric disorders, maternal over- or underweight, current use of medication or psychotropic substances, current alcohol or tobacco consumption, and a protein-restricted diet.

7.2.2 Procedures

Participants in early pregnancy (weeks` gestation: M=9.59, SD=2.15) were invited to our lab at the University of Zurich, to assess sociodemographic and health status using a half-standardized interview. All participants received information about the saliva sampling procedure and were provided with the equipment. The women were asked to collect saliva at home in four week intervals during pregnancy until early postpartum. Each collection included three saliva samples. Samples were taken directly after morning awakening (+0 minutes; t1), which describe the basal cortisol levels, at +30 minutes (t2), and +60 minutes (t3) to assess the awakening response of cortisol and cortisone. Instructions for the saliva collection included to

refrain from excessive sportive activity and alcohol consumption 48 hours prior and no sportive activity, alcohol consumption, caffeine or black tea consumption or chewing gum 24 hours prior to collection. During each sampling period, participants were asked to abstain from teeth brushing and eating, and to reduce drinking to a minimum (Stalder et al., 2016). Participants received a reminder of the sample collection via text message 24 hours beforehand. The exact saliva sampling time and date were annotated by the participants, using written instructions.

7.2.3 Psychological Control Variables

For the mood assessment, a visual analogue scale was administered each time saliva was collected (Klinkenberg et al., 2009). Participants marked how stressed they felt during the last four weeks, on the previous day, and at this moment. Furthermore, participants indicated their anticipated stress level for the current day, and the sleep quality and sleep duration of the past night.

7.2.4 Salivary Cortisol and Cortisone Assay

Saliva was collected by passive drooling using SaliCaps sampling devices (SaliCap, IBL International GmbH, Hamburg, Germany). Participants sent the samples back to our lab after collection. Samples were stored at -20^{o} C until assayed. Salivary cortisol and cortisone were measured using a highly sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. This method enables to distinguish the resembling hormones cortisol and cortisone and prevents cross reactivity (Perogamvros et al., 2009). Salivary cortisol and cortisone were determined at nine measurement points during pregnancy: T1=5-8 weeks` gestation; T2=9-12 weeks` gestation; T3=13-16 weeks` gestation; T4=17-20 weeks` gestation; T5=21-24 weeks` gestation; T6=25-28 weeks` gestation; T7=29-32 weeks` gestation; T8=33-

36 weeks` gestation; T9=37-41 weeks` gestation. Postpartum measures were grouped at T10=0-4 and T11=5-8 weeks postpartum.

7.2.5 Statistical Analyses

Statistical analyses regarding sociodemographic variables were performed using SPSS Version 22.0 (IBM, Armonk, NY). The statistical analyses of cortisol and cortisone were conducted with RStudio 3.1.3 (R Core Team, 2016). Cortisol and cortisone were naturally log transformed due to non-normal distribution; (ln(cortisol+1) and (ln(cortisone+1)). The salivary cortisone-cortisol ratio was calculated by using the following formula: [Cortisone/((Cortisone+Cortisol)*100)], and by utilizing the untransformed cortisol and cortisone values (Benediktsson et al., 1997; Ghaemmaghami et al., 2014; Kajantie et al., 2003). Hierarchical linear models (HLM) with multi-levels for non-independent data were established. To assess non-independence, the intra-class correlation coefficients (ICC) are reported in the result section (Kenny, 1995). The cortisol samples were firstly nested within morning assessment T, which were secondly nested within participants, as displayed in the following formula:

Level 1: $\text{Cortisol pmt} = \alpha 0\ pt + \alpha 1\ pt\ (T\ pmt) + \varepsilon\ pmt$

Level 2: $\alpha 0\ pt = \gamma 0\ p + \theta 0\ pt$

$\alpha 1\ pt = \gamma 1\ p + \theta 1\ pt$

Level 3: $\gamma 0\ p = \gamma 00 + \xi 0\ p$

$\gamma 1\ p = \gamma 10 + \xi 1\ p$

At level 1, the cortisol samples of CAR t, at pregnancy month m, for participant p, is a function of a random intercept of participant and CAR t ($\alpha 0\ pt$), a random slope parameter ($\alpha 1$ pt), which reflects the regression parameter of pregnancy month on cortisol for participant p

and morning assessment t, and a residual variance term (ε pmt). At level 2, the level 1 parameters become the outcomes, whereby a participant`s intercept level at morning assessment t (α0 pt) is a function of the mean intercept over all pregnancy months for participant p (ɣ0 p) and a variance component (θ0 pt), which reflects deviations from a participant`s average across months. Similarly, the random slope for participant p and morning assessment t/ CAR t (α1 pt) is a function of the participants mean slope (ɣ1 p) and a variance component (θ1 pt), reflecting deviations from the mean slope. At level 3, the mean intercept over all pregnancy months for all participants p (ɣ0 p) constitutes a function of the mean intercept over all persons (ɣ00) and a variance component, representing the deviation from this mean (ξ0 p). Similarly, the participant`s mean slope over all months (ɣ1 p), is a function of the slope of the average CAR (ɣ10) and a variance component, representing deviations of the participants of the slope of the CAR (ξ1 p).

The area under the curve with respect to increase (AUCi) was calculated to assess the magnitude of change of cortisol and cortisone during pregnancy (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). As the AUCi with the untransformed cortisol and cortisone values was normally distributed, no log transformation was necessary. In line with Stalder and colleagues (2016), the averaged values of the following control variables were taken into account in each model: age of the participant, BMI of the participant, socioeconomic status (education level, civil status) time of awakening, sleep duration, sleep quality, stress experience over the past four weeks, stress experience on the prior day, momentary stress level, anticipated stress and whether the pregnancy was planned or not. In the result section, only significant outcomes for the control variables are reported. Level of significance was set at $p<.05$ and all analyses were two-tailed.

7.3 Results

7.3.1 Sample Characteristics

The mean age of the participants was 31.25 (*SD* = 3.89, age range: 23 to 42) years and 62 women were nulliparous. Nationality was predominantly Swiss (75%) (see Table 4). For all study participants, gestational age for each measurement point was determined by the medical records obtained after birth.

Table 4: Sample characteristics are displayed with regard to pregnancy and sociodemographic variables (*N* = 100).

Characteristics	
Age [mean ± SD (range)]	31.25 ± 3.89 (23-42)
BMI [mean ± SD (range)]	22.03 ± 2.18 (17.36-29.59)
Nationality [% (n)]	
Swiss	75 (75)
German	21 (21)
Other	4 (4)
Educational level [% (n)]	
University degree	43 (43)
High school leaving qualifications	32 (32)
Annual income in CHF [% (n)]	
> 60,000.00	50 (50)
40,000.00- 60,000.00	26 (26)
< 40,000.00	24 (24)
Civil status [% (n)]	
Married	55 (55)
Cohabitation	44 (44)
Single	1 (1)
Weeks of gestation at T1 [mean ± SD (range)]	
	9.59 ± 2.15 (5- 16.29)
Pregnancy was planned [% (n)]	88 (88)
Primipara [% (n)]	62 (62)
No previous pregnancy loss [% (n)]	89 (89)
Gestational week at delivery [mean ± SD (range)]	39.28 ± 1.44 (34.00-41.71)

7.3.2 Analyses of Non-Independence

The test of whether the cortisol values are dependent within T (T1 toT11) exhibited an ICC of .130 (p<.000). This test for the cortisone values displayed an ICC of .185 (p<.000). For the test of whether the cortisol values are dependent within each participant, the ICC was .138 (p<.000). For the cortisone values an ICC of .168 (p<.000) was shown. Further, the test of whether cortisol values are dependent within a CAR revealed an ICC of .049 (p< 000). This test for cortisone values showed an ICC of .069 (p<.000). Whether the AUCi`s within the CAR cortisol are dependent was tested and an ICC of .029 ($p$=.0004) resulted. The same test for cortisone revealed an ICC of .053 ($p$<.000). Finally, the test, whether the AUCi`s within each participant were dependent displayed an ICC of .148 (p<.000). The same test for cortisone values resulted in an ICC of .196 (p<.000). As non-independence is present in every model, participants cannot be assessed on an individual level, which in turn depicts the naturally nested data and justifies the use of multi-level models.

7.3.3 Basal Cortisol Levels over the Course of Pregnancy and Postpartum

Basal cortisol levels (t1) over the course of pregnancy and postpartum, revealed a quadratic curve (t(785)=-8.682, p<.000). An increase in basal cortisol levels with reference to T1 can be reported for T4 (B=.237, SE=.084, t(750)=2.821, p=.005), T5 (B=.362, SE=.085, t(750)=4.274, p<.000), T6 (B=.382, SE=.085, t(750)=4.469, p<.000), T7 (B=.343, SE=.086, t(750)=3.973 p<.00), and T8 (B=.252, SE=.087, t(750)=2.881, p=.004). Negative associations of the control variables with the course of basal cortisol levels over the course of pregnancy were found for: Stress experience over the past four weeks (B=-.008, SE B=.004, p=.029), quality of sleep (B=-.005, SE B=.003, p=.047), being divorced (B=-.732, SE B=.341, p=.036), apprenticeship as highest educational level (B=-.333, SE B=.162, p=.045), and general qualification for university

entrance as highest educational level (*B*=-.345, *SE B*=.167, *p*=.042). Sleep duration showed a positive association (*B*=.034, *SE B*=.009, *p*=.001).

7.3.4 The Cortisol Awakening Response over the Course of Pregnancy and Postpartum

The CAR cortisol (including t1, t2 and t3) displayed a quadratic course over the course of pregnancy and postpartum (*t*(1696)=-13.680, *p*<.000). A CAR cortisol increase with reference to T1 was detected for T4 (*B*=.237, *SE*=.067, *t*(761)=3.529, *p*<.000), T5 (*B*=.366, *SE*=.067, *t*(761)=5.448, *p*<.000), T6 (*B*=.349, *SE*=.067, *t*(761)=5.192, *p*<.000), T7 (*B*=.357, *SE*=.067, *t*(761)=5.324, *p*<.000), T8 (*B*=.300, *SE*=.067, *t*(761)=4.451, *p*<.000) and T9 (*B*=.218, *SE*=.073, *t*(761)=2.992, *p*=.003). Postpartum cortisol displayed a decrease at T10 (*B*=-.141, *SE*=.068, *t*(761)=-2.065, *p*=.039) (see Figure 3). With regard to the control variables, the stress experience over the past four weeks (*B*=-.010, *SE B*=.003, *p*=.0008) and quality of sleep (*B*=-.005, *SE B*=.002, *p*=.022) were negatively associated with the course of cortisol over pregnancy and postpartum. Sleep duration was positively associated (*B*=.019, *SE B*=.008, *p*=.017).

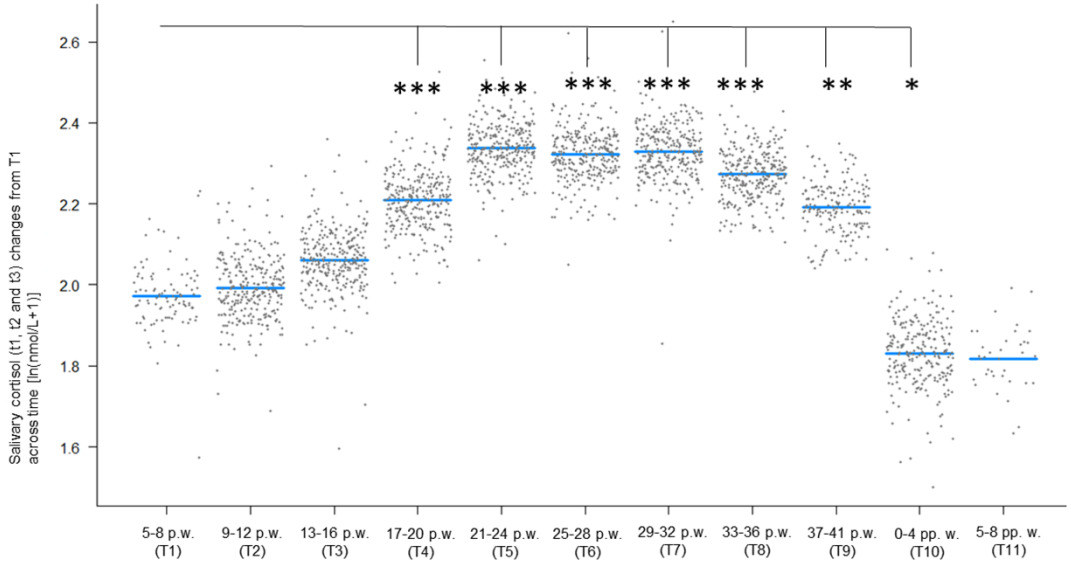

Figure 3: The awakening response of cortisol (CAR; including t1, t2 and t3) over the course of pregnancy (T1-T9) and early postpartum (T10, T11).
p.w. = pregnancy week. pp. w. = postpartum week. *p<.05, **p<.01, ***p<.001.

7.3.5 Cortisol Measured for all Participants at t1, t2 and t3

The CAR was maintained over the course of pregnancy (see Figure 4). Cortisol levels increased from t1 to t2 (t(1506)=10.563, p<.000) and to t3 (t(1506)=5.765, p<.000). The cortisol levels revealed a decrease from t2 to t3 (t(1506)=-10.563, p<.000). With regard to control variables, the stress experience over the past four weeks revealed a negative (B=-.008, $SE\ B$=.003, p=.015) and sleep duration revealed a positive (B=.018, $SE\ B$=.009, p=.046) association.

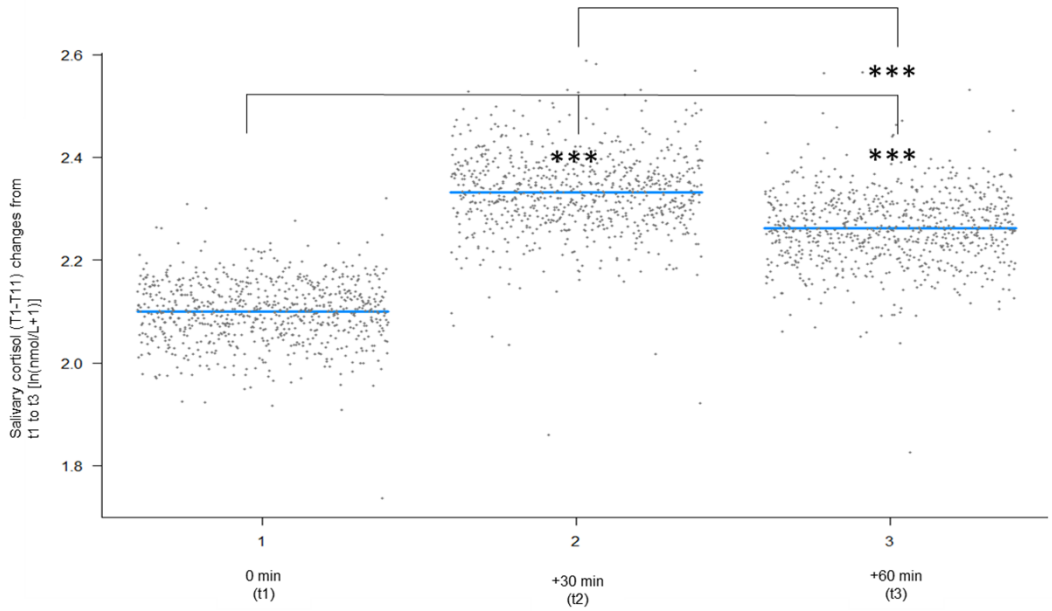

Figure 4: The cortisol awakening response (t1, t2, t3) for all participants over the course of pregnancy. ****p*<.001.

7.3.6 The Area under the Curve for Cortisol Values

A quadratic curve for the AUCi of cortisol over the course of pregnancy and postpartum was revealed ($t(735)=-4.12$, $p<.000$). A significant increase in the values at T8 ($B=2.426$, $SE\ B=1.207$, $t(727)=2.010$, $p<.045$) with reference to T1 was detected (see Figure 5a). Apart from this, no significant change was found. Concerning the control variables, the stress experience over the past four weeks ($B=-0.11$, $SE\ B=.044$, $p=.014$) and sleep duration ($B=-.254$, $SE\ B=.119$, $p=.036$) displayed negative associations. The stress experience on the previous day revealed a positive association ($B=.092$, $SE\ B=.046$, $p=.051$).

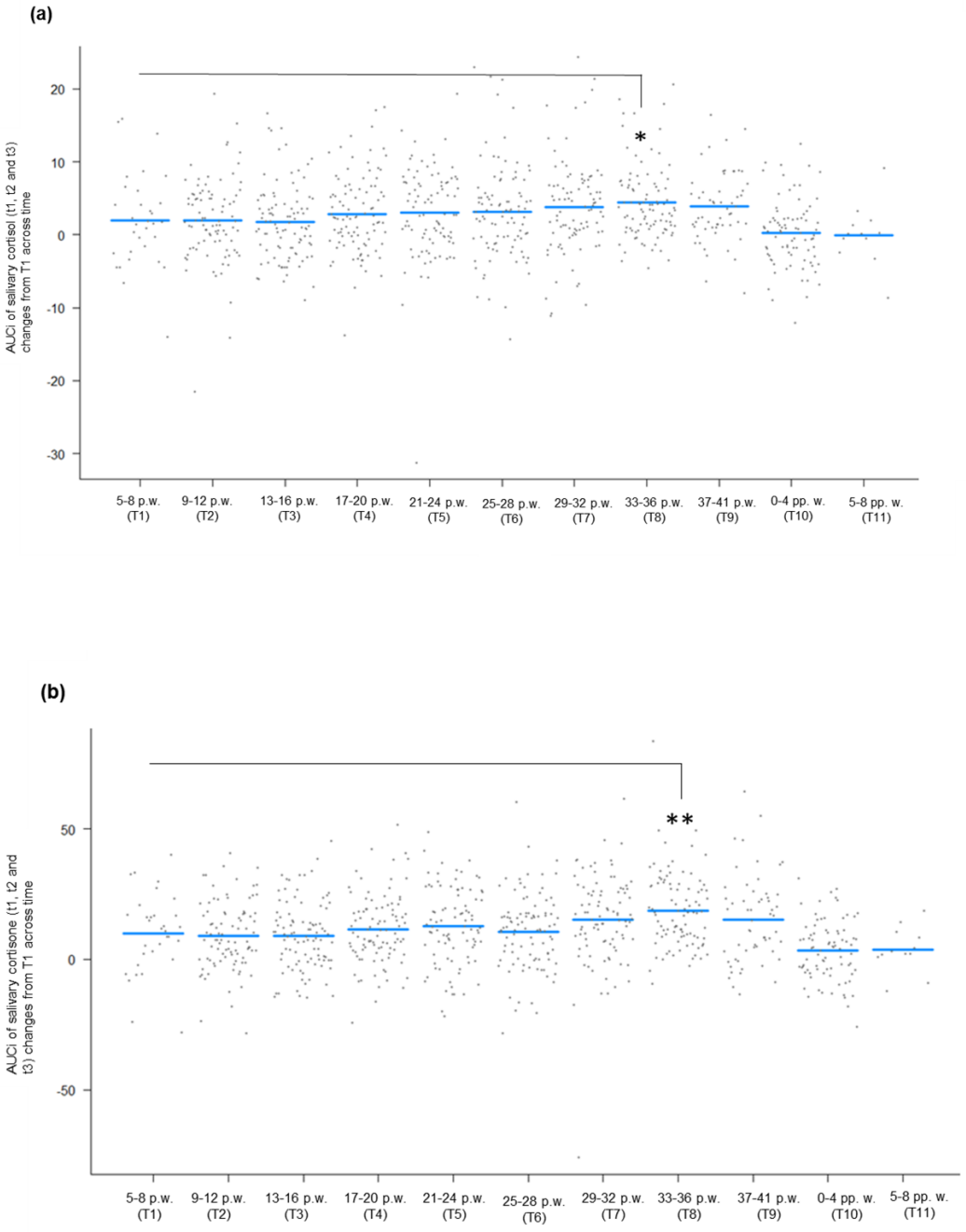

Figure 5: The area under the curve with respect to increase of the cortisol (a) and cortisone (b) awakening responses over the course of pregnancy (T1-T9) and postpartum (T10, T11).
p.w. = pregnancy week. pp. w. = postpartum week. **p*<.05, ***p*<.01.

7.3.7 Basal Cortisone Levels over the Course of Pregnancy and Postpartum

The basal cortisone levels (t1) displayed a quadratic curve over the course of pregnancy and postpartum ($t(785)=-9.002$, $p<.000$). An increase with reference to T1 was detected at T4 ($B=.190$, $SE=.090$, $t(750)=2.111$, $p=.035$), T5 ($B=.316$, $SE=.091$, $t(750)=3.466$, $p=.001$), T6 ($B=.426$, $SE=.093$, $t(750)=4.598$, $p<.000$), T7 ($B=.387$, $SE=.094$, $t(750)=4.106$, $p<.000$), T8 ($B=.469$, $SE=.096$, $t(750)=4.888$, $p<.000$) and at T9 ($B=.521$, $SE=.104$, $t(750)=5.014$, $p<.000$). Basal cortisone levels postpartum were characterized by a non-significant decline with respect to T1. Concerning the control variables, stress during the past four weeks ($B=-.010$, $SE\ B=.004$, $p=.010$) displayed a significant negative association.

7.3.8 The Cortisone Awakening Response over the Course of Pregnancy and Postpartum

The cortisone awakening response (including t1, t2 and t3) over the course of pregnancy and postpartum displayed a quadratic curve ($t(1696)=-12.439$, $p<.000$). An increase in cortisone with reference to T1 was detected for T4 ($B=.144$, $SE=.067$, $t(761)=2.144$, $p=.032$), T5 ($B=.291$, $SE=.067$, $t(761)=4.315$, $p<.000$), T6 ($B=.317$, $SE=.067$, $t(761)=4.709$, $p<.000$), T7 ($B=.364$, $SE=.067$, $t(761)=5.406$, $p<.000$), T8 ($B=.477$, $SE=.068$, $t(761)=7.053$, $p<.000$), and T9 ($B=.463$, $SE=.073$, $t(761)=6.322$, $p<.000$) (see Figure 6). Cortisone at postpartum showed a decrease for T10 ($B=-.262$, $SE\ B=.069$, $t(761)=-3.825$, $p=.0001$) with regard to T1. Concerning the control variables, stress during the past four weeks ($B=-.011$, $SE\ B=.003$, $p=.001$) revealed a negative association.

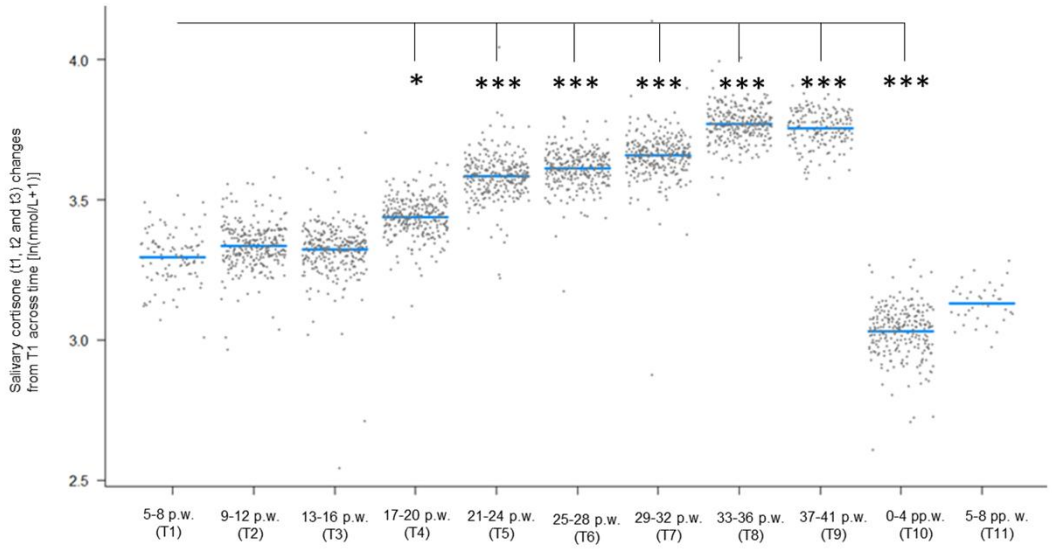

Figure 6: The awakening response of cortisone (CAR; including t1, t2 and t3) over the course of pregnancy (T1-T9) and early postpartum (T10, T11).
p.w. = pregnancy week. pp. w. = postpartum week. *p<.05, ***p<.001.

7.3.9 The Area under the Curve for Cortisone Values

The AUCi for cortisone displayed a quadratic curve over the course of pregnancy and postpartum (t(735)=-4.699, p<.000). Further, an increase in values at T8 (B= 8.971, *SE* B=3.251, t(727)=2.759, p=.006) with reference to T1 was found (see Figure 5b). Concerning the control variables, BMI (B=.958, *SE* B=.485, p=.052) revealed a positive trend and highest educational level being secondary school (B=-13.041, *SE* B=5.589, p=.027) displayed negative association.

7.3.10 The Ratio of Cortisone and Cortisol

The awakening response of the cortisone-cortisol ratio displayed a quadratic curve over the course of pregnancy and postpartum ($t(1692)=4.379$, $p<.000$). A decrease with reference to T1 was detected for T4 ($B=-2.504$, $SE=.779$, $t(761)=-3.214$, $p=.0014$), T5 ($B=-2.374$, $SE\ B=.780$, $t(761)=-3.043$, $p=.0024$), and an increase at T8 ($B=1.761$, $SE\ B=.783$, $t(761)=2.248$, $p=.025$) and T9 ($B=2.909$, $SE\ B=.849$, $t(761)=3.427$, $p=.0006$; Figure 7). Concerning the control variables, sleep duration was negatively associated with the course of the ratio at borderline significance ($B=-.235$, $SE\ B=.118$, $p=.0502$).

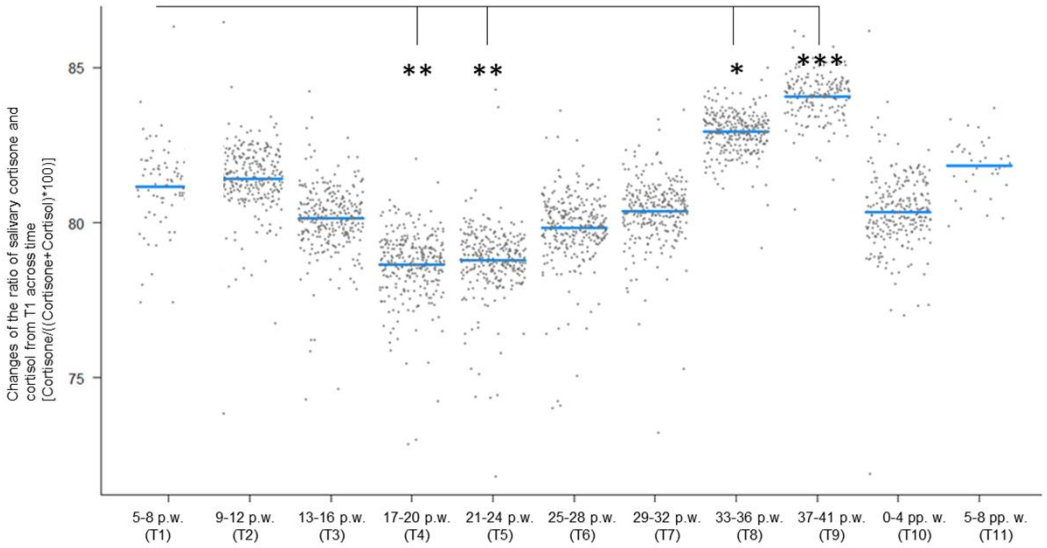

Figure 7: The awakening response of the cortisone-cortisol ratio (CAR; including t1, t2 and t3) over the course of pregnancy (T1-T9) and early postpartum (T10,T11).
p.w. = pregnancy week. pp. w. = postpartum week. *$p<.05$, **$p<.01$, ***$p<.001$.

7.4 Discussion

The study at hand aimed at exploring cortisol, cortisone and their ratio over the course of gestation and postpartum. Especially, studies examining these parameters in early pregnancy seem missing. We showed that basal levels of cortisol and cortisone increased from mid gestation. The awakening response of cortisol and cortisone levels were characterized by an increase from mid until the end of gestation and a decrease postpartum. The examination of the cortisone-cortisol ratio showed a decrease at mid and an increase at late gestation.

The results of the current study revealed a significant increase of cortisol with progressing pregnancy. Basal morning salivary cortisol levels increased from 17-20 weeks` gestation to 33-36 weeks` gestation. Also, basal salivary cortisone levels increased from 17-20 weeks` gestation to 37-41 weeks` gestation. These outcomes corroborate previous findings in the literature. Allolio and colleagues (1990) compared non-pregnant to pregnant subjects and found an increase in salivary cortisol at 25-28 to 37-40 weeks` gestation. However, compared to the current results, Allolio and colleagues (1990) analysed the mean of daily salivary cortisol profiles and the study at hand, found an earlier onset of the increase of basal salivary cortisol levels. DiPietro and colleagues (2011) analysed salivary cortisol in weekly intervals from the 24^{th} until the 38^{th} weeks` gestation. They found an increase in cortisol levels for this period, even though the increase revealed an attenuation at 33 weeks` gestation.

The maintenance of the CAR in the third trimester appears evident (de Weerth & Buitelaar, 2005). The current results support and extent this finding, since the CAR was maintained over the entire course of gestation. Further, it has been assumed that the CAR becomes more attenuated with progressing gestation (Entringer et al., 2010). However, our findings showed an increase in the CAR, as the cortisol values of the CAR were elevated from 17-20 until 37-41 weeks` gestation with reference to early gestation. Nevertheless, values for 25-28 weeks` gestation, 33-36 weeks` gestation and 37-41 weeks` gestation were smaller, but still positively significant, in comparison to the remaining significant values. In spite of the rising CAR with progressing gestation, a slight attenuation from approximately 25 weeks‘

gestation would support the findings from Entringer and colleagues (2010), who compared the CAR at 17 weeks` gestation with the CAR at 31 weeks` gestation. The attenuation of the CAR during pregnancy was also examined with reference to maternal stress. An attenuated CAR was associated with negative life events in early stages of gestation (Obel et al., 2005). Another study reported a trend for a blunted CAR for pregnant women (25-33 weeks` gestation), who took antidepressant medication, in comparison to non-depressed controls (Shea et al., 2007). Interestingly, the current results for the AUCi cortisol only revealed a significant increase at 33-36 weeks` gestation with reference to early gestational stages. This might indicate that, within the current sample, the CAR strengthens over the course of pregnancy.

Due to the absence of longitudinal assessments of salivary 11β-HSD2 activity, the current study considered apart from cortisol additionally cortisone and the ratio between the two markers. An increase in basal cortisone levels and the awakening response of cortisone was detected from 17-20 weeks` gestation to 37-41 weeks` gestation. In a previous study, maternal salivary cortisol and cortisone levels were investigated between 34-36 weeks` gestation to assess the diurnal rhythm of these hormones (Wilson & Thayer, 2016). Elevated cortisone levels in comparison to cortisol levels were found (Meulenberg & Hofman, 1990). It appears critical to consider the activity of 11β-HSD2 in the parotid glands, when salivary cortisol is investigated, since the enzyme`s activity influences the cortisol levels in saliva (Gröschl, 2008; Perogamvros et al., 2012). Meulenberg and Hofman (1990) presumed an increase in salivary 11β-HSD2 activity during gestation because they found an elevated mass ratio of plasma cortisol to salivary cortisol in comparison to the ratio for cortisone. However, the current study revealed first a decrease in the cortisone-cortisol ratio at 17-20 and 21-24 weeks` gestation, followed by an increase at 33-36 and 37-41 weeks` gestation. This supports the hypothesis that the second trimester might represent a time of potentially elevated risk for glucocorticoid over-exposure to (Nierop et al., 2006). A prolonged recovery in the second compared to the third trimester was found, when investigating the salivary cortisol response after a standardized psychosocial stress test (Nierop et al., 2006). This finding would be in line with a metabolic upregulation of salivary 11β-HSD2 activity with progressing gestation. To date, research on

salivary 11β-HSD2 activity has mostly focused on one or two pregnancy trimesters (Ghaemmaghami et al., 2014; La Marca-Ghaemmaghami et al., 2013). However, unlike this work, no study investigated the cortisone-cortisol ratio over the entire course of gestation. This is insofar relevant, as the various stages during pregnancy might differ with regard to the 11β-HSD2 activity in the parotid glands and thereby with the potential maternal glucocorticoid exposure. It appears critical to investigate hormonal alterations during pregnancy not only by using a single point measure every trimester, because the dynamic modifications of the cortisone-cortisol ratio may remain undisclosed.

We recruited our participants via various tools to address a heterogeneous sample, which is representative for the Swiss population. However, our participants revealed high educational levels and high incomes, which might restrict generalizability. Further, future studies are necessary to investigate the potential impact of stress and psychosocial factors on cortisol, cortisone and their ratio. Another limitation of the current work is the indirect assessment of the 11β-HSD2 activity in the parotid glands by using the cortisone-cortisol ratio. As outlined above, even though this procedure is often reported in literature, we did not measure the enzyme activity directly. A strength of the underlying study is the large sample size, as well as the longitudinal design.

To conclude, the present study aimed at examining cortisol, cortisone and their ratio over the course of pregnancy and early postpartum. The results corroborated the rise of cortisol and cortisone from mid gestation and provided new insights, by demonstrating a decrease in the cortisone-cortisol ratio in mid gestation, followed by an increase in late gestation.

PART III:

GENERAL DISCUSSION

8 Summary of the Empirical Findings

The current work unifies the results of two empirical studies considering numerous psychobiological parameters of stress during human gestation. In the first empirical study, we provided novel evidence for psychological work stress variables predicting maternal wellbeing and birth outcome. To be more precise, the scales of work overload, pressure to perform, work disconnect, excessive demands at work, and lack of social recognition, as well as the screening scale of chronic stress of the TICS were utilized in early and late pregnancy and referred to maternal wellbeing, assessed in early pregnancy and postpartum. In addition to the total study population, two subsamples were investigated. We found that diverse aspects of psychological work related stress predicted lowered maternal wellbeing in all examined study groups at various measurement points. With regard to the birth outcome parameters, weeks of gestation at birth, size at birth and birthweight were analysed. Aspects of psychological work stress revealed predictive values for weeks of gestation at birth and birth weight in the total study population. In addition, psychological aspects of work stress predicted size at birth in one of the subsamples.

In the second empirical study, levels of cortisone, cortisol and their ratio, assessed in saliva samples, was recurrently investigated over the course of gestation, beginning in early stages of pregnancy, reaching into the early postpartum period. We found an increase in basal cortisol levels in saliva from mid gestation until late gestation. For basal cortisone levels in saliva, the increase continued until the end of pregnancy. The CAR cortisol was characterized by increasing values from mid gestation until the end of pregnancy and a decrease in the early postpartum period. The awakening response of cortisone displayed the same results. The AUCi of cortisol and cortisone both reached a statistical increase in late stages of pregnancy. The ratio of cortisone and cortisol, which is assumed to represent the 11β HSD2 activity in saliva, revealed interestingly a decrease in earlier stages of pregnancy, followed by an increase in late gestational stages.

8 Summary of the Empirical Findings

In the following chapters, the current results will be integrated into existing literature and discussed with reference towards it. Further, the strengths and limitations of the two empirical studies will be discussed, followed by an outlook for future research.

9 Discussion and Integration of the Empirical Findings

The underlying thesis revealed new findings regarding psychological aspects of work stress during pregnancy predicting maternal wellbeing and birth outcome. These results amplify existing research, as during pregnancy mainly external work related factors (such as long working hours) were considered as potentially stressful. In contrast, psychological aspects of work related stress appeared of less interest. In addition, existing research on work stress during pregnancy focused on birth outcome parameters, leaving maternal wellbeing in comparison appear understudied. Further, research was often conducted in later gestational stages.

The first empirical study of this thesis considered the above mentioned aspects of work related stress during pregnancy and by this aimed at extending work related stress research. Existing literature provided heterogeneous evidence for associations of various aspects of external work related stress and affected birth outcome parameters (e.g., Bonzini et al., 2011; Katz, 2012). Even though, psychological aspects of work related stress were studied to a far lesser extent than external aspects, a few studies considered this factor. High job strain was found to be associated with SGA (Vrijkotte et al., 2009). These results were also found by Brett, and colleagues (1997), but conditioned on working full time or more than 30 hours per week on a high strain job. Further, work dissatisfaction and heteronomy was related to preterm birth (Saurel-Cubizolles et al., 2004). Based on these findings, our study hypotheses were developed in the first empirical study. The results were in accordance with existing research. We refined the current research to that extend that the above mentioned five different aspects of psychological work related factors were analysed with regard to week of gestation at birth, size at birth and birth weight. Further, these associations were investigated in the total study population, a group of women with elevated job strain and a group of women with low income. Associations of aspects of psychological work stress and week of gestation at birth and birth weight were significant in all studied groups. However, psychological aspects of work stress

only predicted size at birth in the elevated job strain group. These results can be considered as homogenous with the existing findings in the literature in terms of that working during pregnancy in special occupational categories may elevate the risk for adverse birth outcomes. However, these categories were so far mainly described as physically demanding (e.g., McDonald et al., 1988). These aspects of work stress in relation to birth outcome in a more vulnerable study population were also analysed by Pinhas-Hamiel and colleagues (1999), who concluded that a stressful working environment with high pressure is associated with adverse birth outcome. Our study group of pregnant women with elevated job strain was characterized by enhanced levels of work related strains and within this group, aspects of increased psychological work stress had a predictive value for size at birth. This seems to demonstrate the necessity to evaluate the overall work related stress level in pregnant women and pay attention to additional work related stressors, which could elevate the risk for adverse birth outcome. Further, this first empirical study also evaluated associations of aspects of psychological work related stress and maternal wellbeing.

It seems evident that chronic stress during pregnancy affects maternal wellbeing (La Marca-Ghaemmaghami & Ehlert, 2015; Robertson et al., 2004). However, if psychological aspects of work stress during pregnancy affect maternal wellbeing remains mainly elusive, as research results were few and contradicting (Adejumo, 2008; Sanguanklin et al., 2014). Our findings revealed that various aspects of psychological work stress were associated with decreased maternal wellbeing. Results appeared more robust for the assessment of psychological work stress and maternal wellbeing in early pregnancy and showed significances among all study groups in comparison to the postpartum measures. By this, our results support and extend those from Sanguanklin and colleagues (2014), who assessed the associations during later gestational stages. We showed that not only psychological work stress in the third trimester of pregnancy predicted maternal wellbeing, but that also early gestational stages should receive increasing attention.

The second empirical study aimed at investigating the glucocorticoids cortisol, cortisone and the cortisone and cortisol ratio, assessed in saliva, over the course of pregnancy and early

postpartum. The aforementioned results demonstrated an increase in cortisol baseline measures in saliva from mid gestation (17-20 gestational weeks) to late gestation (33-36 gestational weeks). These results were also found for basal cortisone levels. An increase was found from mid gestation onwards until the end of gestation (37-40 gestational weeks). The awakening responses of cortisol and cortisone were characterized by an increase from mid gestational stages up to the end of gestation and a decrease in early postpartum. To the best of one's knowledge, no research exists, apart from the study from Allolio and colleagues (1990), which considered cortisol levels in saliva over the course of pregnancy with assessments at every pregnancy month. In the Allolio study, the mean cortisol levels from diurnal assessment in 12 pregnant women were analysed and displayed a steady increase from 25-28 weeks of gestation until the end of pregnancy. Our empirical study revealed an increase as early as 17-20 gestational weeks up to late stages of gestation in the investigated basal salivary glucocorticoids. This extends the findings by Allolio and colleagues (1990), demonstrating an even earlier onset of the increase in cortisol and cortisone. The awakening response of cortisol and cortisone, assessed in saliva samples, upon awakening during pregnancy were mainly analysed at very few time points and often in reference to its potentially stress conditioned alterations (e.g., Bolten et al., 2011; de Weerth & Buitelaar, 2005; Ghaemmaghami et al., 2014; Giesbrecht et al., 2013; Suglia et al., 2010). Interestingly, the assessment of cortisol over the entire course of pregnancy in fundamental research remains understudied. The current study aimed at exploring natural modifications of the glucocorticoids during the entire pregnancy and the early postpartum period. In addition, the activity of salivary 11β-HSD2, determined by the ratio of cortisone and cortisol in saliva, over the entire course of pregnancy with assessments at every pregnancy month, has not been considered in scientific assessment so far. Meulenberg and Hofman (1990), who took saliva samples for the assessment of cortisol and cortisone from 36 pregnant women at four occasions during gestation (14-19 gestational weeks, 20-26 gestational weeks, 27-34 gestational weeks and 35-40 gestational weeks), detected an increase over the course of gestation in both parameters, with the cortisone increase exceeding the cortisol increase. According to the authors, this discrepancy might be attributed to the metabolic

function of 11β-HSD2 in the parotid glands. This would lead to the assumption of an increase of the 11β-HSD2 activity in the parotid glands over the course of gestation. However, the results of the second empirical study with regard to the ratio of cortisone and cortisol, displayed a different outcome. A significant decrease of the ratio was found in the 17-20 and 21-24 weeks of gestation, followed by an increase in 33-36 and 37-40 gestational weeks. This novelty of findings could support the assumption, that the earlier stages of pregnancy might be more vulnerable in comparison to later stages. The protective mechanisms of 11β-HSD2 in the parotid glands do not seem to be available during the early gestational stages. Overall, the current results suggest that there are deviations in the activity of 11β-HSD2 in the parotid glands with regard to the different stages of pregnancy.

10 Strengths and Limitations

The two underlying empirical studies contributed to and extended existing stress research about naturally occurring stress during human gestation. Both studies were included in the broader frame of a longitudinal project. The longitudinal design of the current studies can be considered as a major strength.

In the first study, psychological parameters were assessed in early and late gestational stages and the early postpartum period and analysed in relation to birth outcome and maternal wellbeing. In the second empirical study, the longitudinal design embedded iterated measurement points for the cortisol and cortisone assessment in saliva in four week intervals, starting in early pregnancy and reaching into early postpartum. This provided the opportunity to evaluate the course of the pregnancy and postpartum. Further, the conduct of both studies enabled to assess data mainly in its natural environment. Participants were invited into our laboratory at the University of Zurich, Switzerland at the beginning of the study, where sociodemographic and psychological parameters were collected and the procedure of the longitudinal design was explained. Afterwards, participants conducted the saliva sampling for the assessment of cortisol and cortisone levels at home and filled out additional surveys online. This procedure was utilized to enhance study commitment and to avoid social desirable answering patterns. The final personal appointment with the participants was conducted in the early postpartum period. To minimize effort for the study subjects, the final appointment mainly took place at home at the participant's discretion. These factors contributed to a small number of dropouts and enhanced the subject's willingness to complete their participation.

An additional strength of the empirical studies is represented by the relatively large sample size. Especially, existing studies in the field of endocrine research usually display a much lower number of participants. Our study participants were healthy and characterized by a wide age range. Because of the stringent inclusion criteria, the study population can be considered as relatively homogeneous. We recruited participants via various online platforms,

local gynaecological practices and at the University Hospital in Zurich, Switzerland to address a wide range of study subjects.

However, generalizability might be compromised as the high educational level of the participants, as well as the comparable high income is not representative for the entire Swiss population. This can be considered as a limitation of the underlying studies. To address this point, future research should ensure a more dynamic sample in socioeconomic terms and by this increase generalizability. In addition, only healthy singleton pregnant women were included in the current work. Subjects with medical or psychological conditions or women with multiple pregnancies were excluded. Especially with regard to psychological wellbeing, there appears to exist a difference between singleton and multiple parents during pregnancy and in the early postpartum period, with parents of multiples revealing higher levels of psychological distress (Wenze, Battle, & Tezanos, 2015). Future research should consider multiple subsamples of pregnant women to extent research to the various types of pregnancies. Further, another limitation of the second study addresses the saliva sampling. Participants were kindly reminded via text message one day prior to the collection and received detailed written instructions at the beginning of the study participation. Further, our study subjects wrote down the exact sampling time and date at every measurement point. However, time and date of the sampling, as well as the instructions, for example to abstain from teeth brushing during the sampling time, were not supervised and could therefore constitute the potential for incorrect saliva sampling. Future studies might benefit from objective monitoring devices, as laid out by Stalder and colleagues (2016) even though costs per participant would be enhanced.

11 Outlook, Implications and Conclusions

By investigating human gestation from a psychoendocrine perspective, new insights and amplifications in this research field were provided. The first empirical study contributed to enhanced knowledge about factors of psychological work stress during pregnancy and its predictive value for maternal wellbeing and birth outcome. However, a variety of ensuing research questions resulted, which were beyond the current work to answer. Three measurement points (early and late gestational stages and postpartum) were considered in the first empirical study. However, due to the study design, the second trimester of pregnancy was not incorporated into the analyses of the first study. Future studies should therefore attempt to additionally assess psychological work stress during the second trimester in relation to maternal wellbeing and birth outcome. This would provide a more complete understanding of when psychological work stress constitutes a potential risk for the expectant mother and her unborn child. This in turn could initiate a change of thinking in social manners with regard to more care and protection of working pregnant women. If future research supports this approach, interventions could be developed and preventive methods could be derived. Employees as well as employers could benefit from this knowledge, as potential risks for maternal or foetal adverse health outcomes, as a consequence of elevated psychological work stress, might be reduced.

Regarding the second empirical study, particularly important appears the consideration of the hormones cortisone and cortisol, as well as their ratio, in saliva with respect to potential stress induced changes. To be more precise, every pregnancy month should be investigated to access deeper knowledge about which period of pregnancy is especially vulnerable towards maternal stress exposure. As stress is common in our society and most likely not entirely preventable over the course of gestation, the examination of the cortisone and cortisol ratio appears critical. The above mentioned metabolic processes of the 11β-HSD2 activity in the parotid glands could be derived from this ratio. Literature indicates that stress might dampen the enzyme activity in the parotid glands (Ghaemmaghami et al., 2014). It appears critical to

investigate the exact time intervals during pregnancy with regard to the potential impact of stressful stimuli on the enzyme activity. The protective mechanism of 11β-HSD2 for the unborn child could be supported by instrumental initiatives in relation to its natural biological vulnerability. If the awareness of a biological vulnerability is consolidated, not only health benefits for the expectant mother and her child could results, but further programs for stress management could be developed for pregnant women and be either applied during the vulnerable periods or as a preventive measure beforehand.

To conclude, the current thesis provided novel insights with respect to associations between work related stressors and maternal wellbeing, as well as birth outcome. A better understanding about which psychological factors of work related stress during pregnancy predict maternal wellbeing and birth outcome could be derived from the first empirical study. Further, the adaptation to naturally occurring stress during pregnancy was explored from a psychoendocrine perspective in the second empirical study. The fluctuation of the enzymatic mechanisms became apparent. These findings contributed to a better understanding of the potential vulnerabilities during human gestation. However, numerous questions remain unanswered and further research is necessary to improve and extend the knowledge in this important research field.

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